ASSOCIATION OF FUNCTIONAL DYSPEPSIA WITH DUODENAL EOSINOPHILIA: A PROSPECTIVE CASE CONTROL STUDY

A THESIS SUBMITTED TO BABA FARID UNIVERSITY OF

HEALTH SCIENCES, FARIDKOT (PUNJAB)

IN THE PARTIAL FULFILLMENT

FOR THE AWARD OF DEGREE OF

DOCTORATE OF MEDICINE

IN

GASTROENTEROLOGY

2024

Submitted By

DR. MANISHA KHUBBER

DEPARTMENT OF GASTROENTEROLOGY
DAYANAND MEDICAL COLLEGE AND HOSPITAL
LUDHIANA, PUNJAB

DECLARATION BY THE CANDIDATE

I hereby declare that the thesis entitled **"ASSOCIATION OF FUNCTIONAL DYSPEPSIA WITH DUODENAL EOSINOPHILIA: A PROSPECTIVE CASE CONTROL STUDY"** submitted towards partial fulfillment of the requirements for the award of **D.M. Gastroenterology** degree to BFUHS is a compilation of original work carried out by me under the supervision of **Dr. Omesh Goyal,** Professor, Department of Gastroenterology, **Dr. Ajit Sood,** Professor & Head, Department of Gastroenterology and **Dr. Harpreet Kaur,** Professor, Department of Pathology. No part of this thesis has formed the basis for the award of any degree previously.

_____

DR. MANISHA KHUBBER
Department of Gastroenterology
Dayanand Medical College & Hospital,
Ludhiana, Punjab

Date:

Place:

CERTIFICATE OF SUPERVISORS

This is to certify that the work incorporated in this thesis entitled **"ASSOCIATION OF FUNCTIONAL DYSPEPSIA WITH DUODENAL EOSINOPHILIA: A PROSPECTIVE CASE CONTROL STUDY"** has been carried out by **Dr. Manisha Khubber** under our direct supervision. The data incorporated in this thesis is original and genuine and has been carried out by the candidate herself.

Signature of Supervisor

Dr. Omesh Goyal
Professor
Department of Gastroenterology
DMCH, Ludhiana

Signature of Co-Supervisor

Dr. Ajit Sood
Professor & Head
Department of Gastroenterology
DMCH, Ludhiana

Dr. Harpreet Kaur
Professor
Department of Pathology
DMCH, Ludhiana

Date:

ENDORSEMENT BY THE HEAD OF THE INSTITUTE

This is to certify that the thesis entitled **"ASSOCIATION OF FUNCTIONAL DYSPEPSIA WITH DUODENAL EOSINOPHILIA: A PROSPECTIVE CASE CONTROL STUDY"** is a bonafide research work done by **Dr. Manisha Khubber** under the supervision of **Dr. Omesh Goyal,** Professor, Department of Gastroenterology, **Dr. Ajit Sood,** Professor & Head, Department of Gastroenterology and **Dr. Harpreet Kaur,** Professor, Department of Pathology in this institution.

Principal

Dr. GS Wander
Principal
DMC&H, Ludhiana

Date:

COPYRIGHT

Declaration by the Candidate

I hereby declare that the Baba Farid University of Heath Sciences, Faridkot, shall have the rights to preserve & use this thesis in print or electronic format for academic/study purpose by the research scholar/students.

Signature

Dr. Manisha Khubber
Department of Gastroenterology
Dayanand Medical College & Hospital,
Ludhiana, Punjab

Date:

© Baba Farid University of Health Sciences, Faridkot

Dedicated

to

my Parents

&

Family

ACKNOWLEDGEMENT

First and foremost, I extend my deepest gratitude to Dr.AjitSood, Professor and Head, Department of Gastroenterology, whose extraordinary wisdom, expertise, and mentorship have been the guiding light of my research journey. His remarkable knowledge and insight into the field of gastroenterology is truly unparalleled, and I have been fortunate to benefit from his vast experience. Dr.Sood's exceptional leadership and mentorship have not only shaped the direction of this work but have also inspired me to strive for excellence in my academic pursuits. His dedication to the pursuit of knowledge is contagious, and his commitment to his student's success is unwavering. I am in awe of his exceptional ability to balance his roles as a teacher, researcher, and mentor, and his humility and kindness despite his immense knowledge and achievements are truly remarkable. Dr.Sood's guidance has been instrumental in helping me navigate the complexities of research, and his insightful feedback has been invaluable in shaping the direction of this work. His encouragement and support have meant the world to me, and I am forever grateful for the opportunity to have worked under his guidance. In addition to his academic prowess, Dr.Sood is an exceptional human being, whose compassion, empathy, and kindness have made a profound impact on my life. His ability to connect with people from all walks of life and his willingness to help those in need

are qualities that make him a truly remarkable individual. I am honored to have had the privilege of working with Dr.Sood, and I aspire to emulate his qualities of wisdom, humility, and dedication to the pursuit of knowledge. I am confident that his legacy will continue to inspire generations of researchers and clinicians to come.

I am also deeply indebted to Dr.Omesh Goyal, Professor, Department of Gastroenterology, for his scholarly advice, encouragement, and unwavering support throughout this research endeavour. Sir is one of the most humble professors I have had the privilege of meeting, and his exceptional knowledge and expertise in the field of Gastroenterology have been a constant source of inspiration and guidance. As one of the finest researcher in functional bowel disorders, his skills and expertise have been a valuable asset to this research. His ability to distill complex concepts into simple, understandable terms has been enlightening, and his passion for teaching and mentoring is evident in everything he does. Dr.Omesh Goyal's door has always been open to me, and he has generously shared his time, expertise, and resources to help me navigate the challenges of this research. His encouragement and support have meant the world to me, and I am forever grateful for the opportunity to have learned from him. What strikes me most about Dr.Goyal is his exceptional humility, despite being one of the most knowledgeable and skilled professionals in his field. He is a true role model for anyone aspiring to make a difference

in the world of medicine, and I feel fortunate to have had the opportunity to learn from him. His contributions to this research have been invaluable, and I am so grateful to have had him as a mentor and guide. I feel honored to have been a part of his journey.

I express my sincere appreciation to Dr Harpreet Kaur, Professor& Head, Department of Pathology for his invaluable assistance and support throughout the phases of research. His meticulous attention to detail and collaborative spirit significantly enhanced the quality of research. Working with him has been inspiring and enriching experience.

I would like to express my gratitude to Dr Varun Mehta, Dr Ramit Mahajan, Professors, Department of Gastroenterology, Dr Arshdeep Singh and Dr Yogesh Gupta, Associate Professors Department of Gastroenterology. Their invaluable assistance and encouragement have been significant source of motivation through phases of research.

My gracious thanks to the staff of Endoscopy Department and Research Department DMC&H for helping me thoroughly with the analysis and formatting of this dissertation.

Mom and Dad, thank you for everything. I am blessed to have you as my constant source of motivation and your sacrifices to provide me with the best of everything have Been driving force behind my accomplishments. This

journey and work is a reflection of the values you instilled in me and the love you showered on me. I am always indebted to you.

I am profoundly thankful to my siblings, Sonam and Garvdeep Singh for their constant support.

I would like to thank my friends and colleaguesDr Mudasir Bashir Sheik, Dr Shivam Bansal, for persistent belief in me and your willingness to lend a helping hand. Countless moments of motivation and laughter we shared made this journey not only academically fulfilling but also personally enriching. I am grateful for the memories we created together.

I am very thankful to my seniors Dr Manjeet Goyal, Dr Mayur Luthra, and juniors Dr Tarun Jain, Dr Karanpreet Singh, Dr Raghav Upneja, Dr Ankit Tandon, Dr Arshiya Bhardwaj, Dr Abhinav Gupta, Dr Yogesh, Dr Akash, Dr Anu, Dr Akshay Mehta, Dr Ujjwal Mahajan and Dr Kushdeep for helping me and guiding me.

This thesis is a result of collective efforts and encouragement of many and I am sincerely grateful to each and every one of you for being part of this academic endeavour.

DR. Manisha Khubber

LIST OF ABBREVIATIONS

5 ASA	5 Amino salicylic acid
ACT 1	Active ulcerative colitis 1 trial
ACT 2	Active ulcerative colitis 2 trial
BLI	Blue light imaging
BLI-BRT	Blue light imaging bright
BMI	Body mass index
FC	Fecal calprotectin
FMT	Fecal microbiota transplant
GS	Geboes score
HEMI	Histologic endoscopic mucosal improvement
HEMR	Histologic endoscopic mucosal remission
IBD	Inflammatory bowel disease
LCI	Linked color imaging
MES	Mayo endoscopic score
MH	Mucosal healing
MUAC	Mid upper arm circumference
NHI	Nancy histological index
PMS	Partial mayo score
SCCAI	Simple clinical colitis activity index
TNF	Tumor necrosis factor
Tofa	Tofacitinib
UC	Ulcerative colitis
UCEIS	Ulcerative colitis endoscopic index of severity

WLE	White light endoscopy
WLI	White light imaging

ABSTRACT

Background: Functional dyspepsia (FD) is a chronic upper gastrointestinal disorder that significantly impairs quality of life (QOL). Emerging evidence suggests a potential link between duodenal eosinophilia and FD, though its clinical significance remains under investigation. This study aims to explore the association between duodenal eosinophilia and FD in a northern Indian population.

Aims and Objectives: The primary objective is to determine whether patients with FD have increased eosinophil count in duodenal biopsy compared to healthy controls. Secondary objectives include defining normal duodenal eosinophil count in a healthy northern Indian population and comparing symptom severity and QOL between patients with normal and increased eosinophil counts.

Materials and Methods: This observational, cross-sectional study included 150 patients with FD and 50 age- and sex-matched controls. All subjects underwent esophagogastroduodenoscopy and duodenal biopsy. Eosinophil counts were determined from biopsy samples, and symptom severity was assessed using the SFNDI (short form of Napean dyspepsia index). Data were analyzed to assess the correlation between eosinophil counts, symptom severity, and QOL. Receiver operating characteristic curve and logistic regression analyses were used to evaluate the cut-ff to define normal versus raised duodenal eosinophil count.

Results: The mean age of patients was 35 years, with a predominance of females (62%). A cutoff of 12 duodenal eosinophils per hpf was derived from healthy control population with the area under the ROC curve of 0.73 (95% CI: 0.66-0.80). Duodenal eosinophilia was significantly higher in patients with FD compared to controls ($p < 0.001$). 64.67% of cases with FD had raised duodenal eosinophilic count. Raised duodenal eosinophil counts were strongly associated with increased symptom severity and poor QOL. Patients with postprandial distress syndrome (PDS) and with retrosternal discomfort had significantly higher duodenal eosinophil counts. Higher rate of eosinophilic degranulation was seen in cases with raised duodenal eosinophil count reflecting activation of eosinophils in FD patients.

Conclusion: In patients with FD, duodenal eosinophilia is significantly more common in PDS subgroup, and those with retrosternal discomfort. Raised duodenal eosinophil counts were strongly associated with increased symptom severity and poor QOL. These findings highlight the potential role of eosinophils in the pathophysiology of FD, suggesting that duodenal eosinophil counts could serve as a biomarker for diagnosis and treatment stratification. Further studies are needed to confirm these results and evaluate the therapeutic implications.

Keywords: Functional dyspepsia, duodenal eosinophil, dyspepsia.

TABLE OF CONTENTS

S. NO.	TOPIC	PAGES
I	INTRODUCTION	1-11
II	AIMS AND OBJECTIVES	12
III	REVIEW OF LITERATURE	13-40
IV	MATERIALS AND METHODS	41-44
V	OBSERVATIONS AND RESULTS	45-74
VI	DISCUSSION	75-85
VII	SUMMARY AND CONCLUSIONS	86-89
VIII	BIBLIOGRAPHY	90-99
	APPENDICES – I - PROFORMA - MASTER CHART	
	APPENDICES – II - APPROVAL LETTER OF THESIS PLAN - THESIS PLAN	

LIST OF TABLES

Table no.	Title	Pages
1	Receiver operating characteristic curve of normal duodenal eosinophil count for healthy population	46
2	Comparison of duodenal eosinophil (/hpf) between cases and controls	47
3	Age Distribution of Cases	48
4	Association of duodenal eosinophil count (/hpf) with age	49
5	Descriptive statistics of dyspepsia subgroups symptom score(SS) in cases.	50
6	Descriptive statistics of subgroup score in cases	53
7	Correlation of Duodenal eosinophil count with various parameters in cases	55
8	Association of dyspepsia subgroups symptom score with duodenal eosinophil in cases	59
9	Association of various parameters with duodenal eosinophil in cases	60
10	Comparison of degranulation between cases and controls	63
11	Association of duodenal eosinophil with degranulation in Cases	64
12	Association of dyspepsia subgroups Symptom Score with degranulation in cases	65
13	Association of duration of dyspepsia and SFNDI QOL in cases with or without degranulation	66
14	Association of degranulation with duodenal eosinophil in cases	68
15	Comparison of co-morbidities and medications between cases and controls	69
16	Association of duodenal eosinophil with comorbidities and medications in Cases	70

17	Association of co-morbidities and medications with raised and normal duodenal eosinophil in cases	71
18	Correlation of gastric eosinophils with various parameters in cases	72
19	Univariate logistic regression to find out significant risk factors of raised duodenal eosinophil in cases	73
20	Multivariate logistic regression to find out significant risk factors ofraised duodenal eosinophil in cases	74

LIST OF FIGURES

1	Receiver operating characteristic curve of normal duodenal eosinophil count for healthy population	46
2	Comparison of duodenal eosinophil (/hpf) between cases and controls.(non-parametric variable, Box-whisker plot)	47
3	Age Distribution of Cases	48
4	Association of duodenal eosinophil count (/hpf) with age.(non-parametric variable, Box-whisker plot)	49
5.1	Descriptive statistics of EPS, PDS and overlap Symptom Score	50
5.2	Descriptive statistics of duration of dyspepsia (years)	51
5.3	Descriptive statistics of SFNDI QOL	51
5.4	Descriptive statistics of gastric eosinophils (%)	52
5.5	Descriptive statistics of PPI duration	52
6	Descriptive statistics of subgroup variables in cases	54
7.1	Correlation of EPS Symptom Score with duodenal eosinophil count (/hpf)	55
7.2	Correlation of overlap Symptom Score with duodenal eosinophil count (/hpf)	56
7.3	Correlation of PDS Symptom Score with duodenal eosinophil count (/hpf)	56
7.4	Correlation of retrosternal discomfort Symptom Score with duodenal eosinophil count (/hpf)	57
7.5	Correlation of SFNDI QOL with duodenal eosinophil count (/hpf)	57
7.6	Correlation of gastric eosinophils (/hpf) with duodenal eosinophil count (/hpf) in cases	58
8	Association of dyspepsia subgroups Symptom Score with duodenal eosinophil in cases	59

9.1	Association of dyspepsia duration with duodenal eosinophil in cases (non-parametric variable, Box-whisker plot)	60
9.2	Association of SFNDI QOL with duodenal eosinophil in cases	61
9.3	Association of gastric eosinophils with duodenal eosinophil in cases	61
9.4	Association of PPI duration with duodenal eosinophil in cases.(non-parametric variable, Box-whisker plot)	62
10	Comparison of degranulation between cases and controls	63
11	Association of duodenal eosinophil with degranulation in Cases.(non-parametric variable, Box-whisker plot)	64
12	Association of dyspepsia subgroups Symptom Score with degranulation in cases	65
13.1	Association of duration of dyspepsia with degranulation in cases.(non-parametric variable, Box-whisker plot)	66
13.2	Association of SFNDI QOL with degranulation in Cases	67

BABA FARID UNIVERSITY OF HEALTH SCIENCES
Sadiq Road, Faridkot-151203 (Pb.) INDIA
Phone No. 91-1639-256253 and Fax No. 91-1639-256234
Website: www.bfuhs.ac.in, Email: bfuhspc@gmail.com
(BFUHS – UNIVERSITY LIBRARY- Coordinator APC)

11-BFUHS (LI-PC-134)24/1186 Dated: September 25, 2024

Plagiarism Verification Certificate

Title of the thesis: **Association Of Functional Dyspepsia With Duodenal Eosinophilia : A Prospective Case Control Study.**

Total Page: **113 (One Hundred and Thirteen only) eFSPC (eFile Sent for Plagiarism Check)**

Researcher: **Manisha Khubber**

Supervisor: **Dr. Omesh Goyal**

Research Centre: **Dayanand Medical College & Hospital, Ludhiana**

This is to report that the above thesis was scanned for similarity detection. Process and outcome is given below:

DID:	**2348788**	Software used:	**DRILLBIT**
Date:	**September 25, 2024**	Similarity Index:	**8% (Below 10 Percent)**

Total words count: **23485 (eFSPC)**

The complete report is submitted for review by the Supervisor and Co-Supervisor.

University Coordinator/
Name & Signature (Checked by)
(From University library)

The complete report of the above thesis has been reviewed by the undersigned. (Check Box)

☐ The similarity index is below accepted norms.

☐ The similarity index is above accepted norms, because of the following reasons:

1.....
2.....
3…….....
4…….....
5……

The thesis may be considered for the award of degree. (Relevant documents attached).

Research Scholar **Co-Supervisor** **Supervisor**

INTRODUCTION

Dyspepsia, in general, is a term classically used to describe symptoms referable to the upper gastrointestinal tract. The term dyspepsia was first coined in the eighteenth centuary. Alarm features of dyspepsia like anemia, dysphagia, unintended weight loss GI bleed or abdominal mass warrant investigation to exclude organic diseases like gastrointestinal neoplasia. After organic pathologies have been ruled out by esophagogastroduodenoscopy and other necessary investigations, the patient is labelled as functional dyspepsia. FD can further be subdivided into those with predominant epigastric pain (epigastric pain syndrome, EPS) or early satiety/post prandial fullness (post-prandial distress syndrome, PDS).[1] Refinement of this definition has sought to exclude patients with heartburn and regurgitation, and have culminated in the Rome IV criteria which specifically defines dyspepsia as troublesome epigastric pain or burning, early satiety or post prandial fullness within the past 3 months with symptom onset at least 6 months earlier in the absence of any organic or structural disease. Due to lack of specific biomarkers, ROME IV criteria are widely used to diagnose functional dyspepsia. [2]

FD is a common clinical entity throughout the world irrespective of geographical locations and ethnicities, with a reported prevalence of 10-30% worldwide, and an annual incidence of 1–3%. The pathophysiology of FD is largely undetermined and satisfactory treatment is not available. Hence, the patients usually undergo repeated medical examinations leading to a substantial burden on healthcare resources. Even though no association has been seen between functional dyspepsia and decreased survival, it is significantly associated with a decreased quality of life and a considerable burden to the healthcare system as well as the patients in terms of reduced opportunity costs. [3,4]

In general, physicians ascribe pathology to symptoms and treatment is prescribed based on the respective pathology underlying the disease. Hence functional gastrointestinal disorders are often perceived to be as difficult to treat. However, as advances are made in attempting to understand the underlying mechanisms FGIDs, there is increasing evidence suggesting a link to localized derangements in the GI-neural axis, including motor and sensory abnormalities, neuro-immunodysregulation and enhanced sensitivity to luminal contents. [5] There are few cost-effective and simple tests that can identify dysmotility in vivo and whilst endoscopy is often performed in these patients, biopsy is not routine to identify subtle pathology changes. [6]

Functional dyspepsia involves a complex interplay of gastrointestinal, immunological, and neurological system disorders, with growing data underscoring the essential involvement of eosinophils in these mechanisms. Eosinophils, historically linked to allergic reactions[7], have lately been related to many gastrointestinal illnesses, including functional dyspepsia (FD).[8] Their existence in the duodenum, especially amid low-grade inflammation, has been associated with many mechanisms contributing to the symptoms of functional dyspepsia, including visceral hypersensitivity, pain, early satiety, dysbiosis, and post-infectious alterations. [9-13]

Eosinophils and Visceral Hypersensitivity

Visceral hypersensitivity is a defining characteristic of functional dyspepsia, greatly contributing to the discomfort and agony reported by patients. It is marked by an excessive reaction to typical gastrointestinal stimuli, such as the consumption of food. Eosinophils' involvement in visceral hypersensitivity is gaining recognition, especially regarding their interactions with other immune cells and the neurological system.[14]Eosinophils play a significant role in visceral hypersensitivity by releasing pro-inflammatory mediators, such as cytokines interleukin-5 (IL-5), interleukin-13 (IL-13), and eotaxins, which recruit and activate more eosinophils and mast cells in the

duodenum.[15] These mediators can directly stimulate nociceptive neurons, resulting in an amplified pain response. IL-5 has been demonstrated to enhance the survival and activation of eosinophils, hence perpetuating inflammation and sensitizing the gastrointestinal tract to stimuli. [16]

Furthermore, eosinophil-derived neurotoxin (EDN) and eosinophil cationic protein (ECP) are recognized for compromising the epithelial barrier, resulting in heightened permeability and facilitating the infiltration of luminal antigens into the gut wall, where they can stimulate sensory neurons. This mechanism is further intensified by the secretion of histamine and tryptase from mast cells, which are frequently located near eosinophils in the duodenal mucosa. The resultant inflammation and nerve sensitization contribute to the increased visceral sensitivity noted in FD patients. [17]

Eosinophils and Pain Mechanisms

The pain associated with FD is believed to be complex, with eosinophils playing a crucial role via many pathways. Eosinophils facilitate pain by the release of cytotoxic granules that harm the intestinal mucosa and the production of cytokines that amplify the inflammatory response. A primary process entails the activation of transient receptor potential (TRP) channels, specifically TRPV1 and TRPA1, on sensory neurons. These channels are recognized for mediating pain and are increased in the presence of eosinophil-derived mediators such as leukotrienes and reactive oxygen species (ROS). [18] The activation of TRP channels induces the production of neuropeptides, including substance P and calcitonin gene-related peptide (CGRP), which further enhance the pain signal. [19]

Furthermore, eosinophils have demonstrated interaction with the enteric nervous system, specifically via the secretion of neurotrophins such as nerve growth factor (NGF), which facilitates neuron development and sensitization. This relationship not only heightens the

gut's sensitivity to nociceptive stimuli but also exacerbates the chronicity of pain in functional dyspepsia. The persistent activation of these pathways may result in chronic pain, rendering even routine physiological functions, such as digestion, uncomfortable for the patient. [20]

Eosinophils and Early Satiety

Early satiety, the feeling of fullness after ingesting a minimal quantity of food, is a prevalent symptom of functional dyspepsia (FD), especially in the postprandial distress syndrome (PDS) subtype. Eosinophils are involved in this symptom by influencing gastric motility and the signaling pathways that govern appetite and fullness. Eosinophils in the duodenum can secrete mediators that disrupt the normal motility of the stomach and small intestine. IL-13 has been demonstrated to modify smooth muscle function, resulting in delayed stomach emptying and diminished gastric accommodation, both of which contribute to early satiety. Eosinophils can also affect the secretion of gastrointestinal hormones, including cholecystokinin (CCK) and ghrelin, which are essential for hunger and satiety regulation. [21,22]

The relationship between eosinophils and the enteric nervous system is pertinent in this situation. Eosinophil degranulation may activate local neuronal circuits that convey signals of satiety to the brain, despite the absence of physical fullness in the stomach. The combination of brain sensitization and decreased stomach motility leads to the typical early satiety observed in patients with functional dyspepsia. [23]

Eosinophils and dysbiosis

Dysbiosis, the imbalance of gut microbiota, is increasingly acknowledged as a significant component in functional dyspepsia (FD). Eosinophils are thought to affect dysbiosis via their interactions with the gut microbiota and their function in regulating immune responses. Eosinophils in the gastrointestinal tract can induce the secretion of antimicrobial peptides and reactive oxygen species (ROS), hence

modifying the gut microbiota composition. Eosinophil-derived mediators, such as eosinophil peroxidase (EPO) and eosinophil cationic protein (ECP), can directly influence microbial populations by establishing an unfavorable environment for specific bacterial species. This disturbance in microbial equilibrium can exacerbate symptoms of functional dyspepsia by fostering inflammation and modifying gastrointestinal Moreover, eosinophils may indirectly impact dysbiosis by modulating epithelial barrier function. Enhanced mucosal permeability facilitates the transfer of bacteria and bacterial compounds into the lamina propria, potentially eliciting an immunological response. This may result in additional recruitment of eosinophils, establishing a feedback loop that sustains inflammation and dysbiosis. [24]

Eosinophils and Post-Infectious Functional Dyspepsia

Post-infectious functional dyspepsia (PI-FD) arises subsequent to an acute gastrointestinal infection, resulting in the persistence of persistent dyspeptic symptoms long after the resolution of the initial infection. Eosinophils play a pivotal role in the progression of PI-FD by mediating the immunological response to infections. In the course of an acute infection, the gut mucosa undergoes inflammation, and eosinophils are mobilized to the affected area as a component of the body's immune response. In certain individuals, this inflammatory reaction fails to completely dissipate, resulting in chronic low-grade inflammation. [14]Eosinophils, through the secretion of cytokines like IL-5 and IL-13, can sustain this inflammatory state by enhancing the survival and activation of additional immune cells, including mast cells and Th2 lymphocytes. The continual presence of eosinophils in the duodenum post-infection may result in enduring alterations to the gut's sensory and motor capabilities. Eosinophil degranulation may compromise the epithelial barrier, resulting in heightened permeability and persistent immunological activation. The persistent inflammation and compromised barrier function can intricately engage with the enteric nervous system, exacerbating the chronic symptoms seen in individuals with PI-FD. [25]

Neuronal Mechanisms Associated with Eosinophils

Eosinophils profoundly influence the enteric nervous system (ENS), which governs gut motility, secretion, and blood flow, hence contributing to the pathophysiology of functional dyspepsia (FD). Eosinophils can engage with the enteric nervous system via direct contact with nerve fibers and by secreting neuroactive chemicals. [16] The link between eosinophils and the vagus nerve is a crucial mechanism in regulating gastrointestinal activities. Eosinophils can secrete neurotrophins such as nerve growth factor (NGF), which facilitate the proliferation and sensitization of nerve fibers. This sensitization may result in an amplified reaction to typical physiological cues, leading to symptoms including discomfort, bloating, and early satiety. Furthermore, eosinophils can regulate the function of the enteric nervous system by secreting cytokines and chemokines that affect the migration and activation of additional immune cells. The secretion of eotaxin can recruit more eosinophils to the inflammatory site, enhancing the immune response and increasing gastrointestinal sensitivity.[16] The relationship between eosinophils and the enteric nervous system (ENS) is associated with the activation of transient receptor potential (TRP) channels on sensory neurons. Leukotrienes, produced from eosinophils, can activate TRPV1 and TRPA1 channels, resulting in the production of neuropeptides such as substance P and CGRP, which amplify pain signals and contribute to the visceral hypersensitivity associated with functional dyspepsia. [26]

Epithelial Integrity and Permeability

Eosinophils play a direct role in compromising epithelial integrity and enhancing intestinal permeability, both of which are essential elements in the pathophysiology of FD. The epithelial barrier is crucial for sustaining gut homeostasis, obstructing the ingress of luminal antibodies, and modulating immune responses. Eosinophils induce epithelial barrier dysfunction by releasing cytotoxic granules, including major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN). These proteins can harm

epithelial cells, resulting in heightened permeability and permitting antigens to infiltrate further into the gut wall. The disruption of the barrier may elicit an immunological response, subsequently attracting eosinophils and sustaining the inflammatory cycle. Enhanced permeability also promotes the transport of bacteria and bacterial products, including lipopolysaccharides (LPS), into the lamina propria, where they can stimulate Toll-like receptors (TLRs) on immune cells. This activation may result in the synthesis of pro-inflammatory cytokines, including IL-1β and TNF-α, which exacerbate epithelial barrier degradation and perpetuate the chronic inflammation observed in FD. [27,28] The integrity of the epithelial barrier is governed by tight junction proteins, including occludin and claudins, which govern the translocation of substances between epithelial cells. Eosinophils can compromise tight junctions by secreting cytokines such as IL-13, which diminishes the expression of these proteins, resulting in increased intestinal permeability and a "leaky gut. [23]

Eosinophils and Anxiety/Stress Reactions

A well-established correlation exists between functional dyspepsia and psychological factors, including worry and stress, with eosinophils thought to mediate this association. The gut-brain axis, characterized by bidirectional communication between the central nervous system (CNS) and the gastrointestinal tract, serves as a crucial conduit through which stress can affect gut function and vice versa.Eosinophils may impact the gut-brain axis by secreting neuroactive compounds that alter both the enteric nervous system and central nervous system. Eosinophils can synthesize neurotrophins such as NGF, which sensitizes the enteric nervous system (ENS) and modulates stress responses through interactions with the hypothalamic-pituitary-adrenal (HPA) axis. This interaction may elevate cortisol production, a stress hormone that can further impair gut function and exacerbate FD symptoms. [29,30] Furthermore, eosinophils may contribute to the onset of anxiety and depression via their influence on gastrointestinal inflammation. Chronic low-grade inflammation, observed in FD, has

been associated with modifications in neurotransmitter systems, including serotonin and dopamine, which play a role in mood regulation. Eosinophils' release of pro-inflammatory cytokines might influence the synthesis and metabolism of neurotransmitters, potentially resulting in mood disorders that aggravate FD symptoms. [26,31] Stress may also boost eosinophil activity in the gastrointestinal tract. Research indicates that stress can elevate the secretion of eotaxin, a chemokine that attracts eosinophils to the gastrointestinal tract. This recruiting may exacerbate local inflammation and aggravate FD symptoms during stressful periods. Eosinophils serve as mediators of gut inflammation and may also connect stress to the worsening of functional dyspepsia symptoms. [23,32]

The eosinophil-mast cell axis in functional dyspepsia

The connection between eosinophils and mast cells, referred to as the eosinophil-mast cell axis, is pivotal in the pathogenesis of FD. Both cell types are crucial components of the immune response and are frequently located in close proximity inside the gastrointestinal mucosa, especially in the duodenum. Their synergistic interaction intensifies the inflammatory milieu and adds to the diverse symptoms linked to FD.

1. Bidirectional activation:Eosinophils and mast cells mutually stimulate one another via the secretion of diverse mediators. Eosinophils emit major basic protein (MBP) and eosinophil cationic protein (ECP), which can activate mast cells to secrete histamine, tryptase, and other pro-inflammatory mediators. Mast cells, in contrast, secrete cytokines such as IL-4 and IL-5 that facilitate the recruitment, activation, and persistence of eosinophils.This bidirectional stimulation enhances the inflammatory response, resulting in heightened vascular permeability, smooth muscle contraction, and mucosal injury, all of which exacerbate the symptoms of FD. [37]

2. Enhancement of inflammation:Products produced from eosinophils can augment mast cell degranulation, resulting in the increased release of pro-inflammatory mediators. This establishes a loop of continuous inflammation, notably observed in patients with enduring symptoms despite intervention. The degranulation of mast cells releases chemokines such as CCL5 (RANTES), which attract additional eosinophils to the site of inflammation, hence prolonging the inflammatory cycle.

3. Contribution to Visceral Hypersensitivity:Mast cells secrete histamine and proteases, which can hypersensitive sensory neurons and enhance pain perception. Eosinophils can exacerbate this process by releasing neurotoxins and cytokines, resulting in the heightened pain responses seen in FD. The closeness of eosinophils and mast cells to nerve fibers in the gastrointestinal mucosa promotes sensitization, directly connecting the immune response to the neurological system's regulation of gut function.

4. Effect on Gastrointestinal Motility:The eosinophil-mast cell axis additionally affects gastrointestinal motility. Eosinophils influence smooth muscle function by releasing cytokines such as IL-13, whereas mast cells secrete histamine and tryptase, which can interfere with proper peristalsis. This motility disruption is linked to symptoms such as early satiety and bloating, prevalent among FD patients.

5. Clinical Significance:Comprehending the eosinophil-mast cell axis reveals prospective pathways for targeted treatments in FD. Interventions designed to stabilize mast cells or diminish eosinophil recruitment and activation may alleviate the chronic inflammation and hypersensitivity associated with FD. Mast cell stabilizers or eosinophil migration inhibitors, such as CCR3 antagonists, may be investigated as therapeutic alternatives.[31,35,37,38]

Interactions of Eosinophils with Other Immune Cells

Eosinophils are intricately linked to Th2 cells, a subset of T-cells pivotal to the immune response in allergy diseases and several inflammatory processes. Th2 cells secrete cytokines including IL-4, IL-5, and IL-13, which are essential for the recruitment, activation, and survival of eosinophils. IL-4 and IL-13 are crucial for the development of naïve T-cells into T-helper 2 (Th2) cells and for facilitating the immunoglobulin E (IgE) class switch in B-cells, potentially resulting in mast cell activation and subsequent eosinophil recruitment. The interaction between eosinophils and Th2 cells enhances the inflammatory response in the gastrointestinal tract, exacerbating the chronic inflammation seen in FD. Regulatory T-cells (Tregs) also contribute to the modulation of this inflammation. Tregs are recognized for their role in sustaining immunological tolerance and inhibiting excessive immune reactions. In the context of FD, an imbalance may exist between Tregs and Th2 cells, resulting in unregulated eosinophil activation. Comprehending this equilibrium may be essential for formulating therapies intended to restore immunological homeostasis in FD.[36,39,40]

Lifestyle, Environmental Factors, and Eosinophils

Allergens : Dietary variables can markedly affect eosinophil activity and the intensity of FD symptoms. Specific foods may provoke or intensify eosinophil activation and the related inflammatory response. Eosinophils are intimately linked to allergic reactions, and dietary allergens can provoke eosinophil activation in predisposed individuals. Recognizing and evading particular dietary triggers like gluten, dairy, or other allergens may mitigate eosinophil-mediated inflammation in patients with FD.[32,42]

High-fat diets: Diets rich in fat have been demonstrated to enhance intestinal permeability and foster inflammation. High-fat meals might intensify eosinophilic inflammation by enhancing the accessibility of luminal antibodies that penetrate the gut barrier, thereby eliciting an immunological response. [43]

Dietary Fiber: Conversely, fiber-rich diets may aid in regulating intestinal inflammation by fostering the proliferation of beneficial bacteria that generate short-chain fatty acids (SCFAs). Short-chain fatty acids have anti-inflammatory characteristics and may modulate eosinophil activity, potentially alleviating symptoms in functional dyspepsia.[44,45]

Ecological Stimuli :Environmental variables, such as exposure to pollution, illnesses, and stress, might affect eosinophil activity and contribute to the onset or worsening of FD symptoms.Airborne contaminants and environmental allergens can activate eosinophils in both the respiratory and gastrointestinal tracts. This is especially pertinent for those with coexisting allergic conditions, such as asthma or allergic rhinitis, when eosinophil activation is already elevated.Gastrointestinal infections, particularly those induced by bacteria such as Helicobacter pylori, can elicit an immunological response resulting in the recruitment and activation of eosinophils. In certain instances, the infection may act as a stimulant for the onset of FD, especially in post-infectious scenarios.[46,47]

AIM AND OBJECTIVES

Primary objective:

1. To determine whether patients with functional dyspepsia have increased eosinophil count in duodenal biopsy compared to healthy controls

Secondary objectives:

1. To define normal duodenal eosinophil count in healthy Northern Indian population.
2. To compare symptoms severity and quality of life using SFNDI QOL questionnaire between patients with normal duodenal eosinophil count and those with increased eosinophil counts

REVIEW OF LITERATURE

Molecular Mechanisms and Eosinophils in Functional Dyspepsia

The molecular relationship between eosinophils and several immunological and neurological pathways is intricate, involving numerous essential receptors, cytokines, and signaling molecules.

1. Receptors and Signaling Pathways:
    a) CCR3 and Eotaxin: The recruitment of eosinophils to the gastrointestinal tract is predominantly facilitated by the interaction between eotaxin and the CCR3 receptor on eosinophils. This interaction is essential for the migration of eosinophils to inflammatory areas and is increased in patients with FD. [33]
    b) TRPV1 and TRPA1 Channels: Mediators produced from eosinophils, including leukotrienes, can stimulate these ion channels on sensory neurons, resulting in pain and hypersensitivity. [26]
    c) Toll-like Receptors (TLRs): Eosinophils can stimulate TLRs on epithelial and immunological cells, resulting in the secretion of pro-inflammatory cytokines that intensify gut inflammation and compromise epithelial integrity. [34]
2. Cytokines and chemokines:
    a) Interleukin-5 (IL-5): This cytokine is essential for the proliferation, differentiation, and activation of eosinophils. IL-5 facilitates eosinophil longevity and amplifies their inflammatory response, hence exacerbating the chronic inflammation characteristic with FD. [15]
    b) Interleukin-13 (IL-13): IL-13, produced by eosinophils and various immune cells, is instrumental in compromising epithelial integrity. It regulates tight junction proteins, resulting in enhanced gut permeability, a critical element in the development of FD. [15]

c) Eotaxin: A chemokine chiefly accountable for the recruitment of eosinophils to the gastrointestinal tract. Eotaxin levels are heightened in patients with FD, coinciding with augmented eosinophil infiltration and inflammation in the duodenum. [35]

d) Tumor necrosis factor-alpha (TNF-α): Released after eosinophil degranulation, TNF-α facilitates inflammation by stimulating the synthesis of additional pro-inflammatory cytokines and compromising the epithelial barrier. [23]

3. Reactive Oxygen Species (ROS) and Nitric Oxide (NO):Eosinophils produce reactive oxygen species (ROS), which serve a dual function in the gastrointestinal tract. Although they aid in pathogen eradication, excessive reactive oxygen species (ROS) can cause tissue damage, exacerbating inflammation and increasing gut hypersensitivity. Nitric oxide, synthesized by diverse cells such as eosinophils, might influence gastrointestinal motility and contribute to the dysmotility seen in functional dyspepsia (FD).

4. Neurotrophins and Growth Factors :Eosinophils synthesize NGF, which enhances the survival and sensitization of neurons in the enteric nervous system (ENS). This sensitization significantly contributes to the visceral hypersensitivity and persistent pain observed in FD patients. [36]

Prolonged Consequences and Persistence

1. Persistent Inflammation and Disease Advancement: Chronic low-grade inflammation mediated by eosinophils and the eosinophil-mast cell axis has consequences that extend beyond the immediate manifestations of functional dyspepsia (FD). Chronic inflammation may result in structural modifications in the gastrointestinal tract, including fibrosis, which could subsequently affect gut function over time.
2. Fibrosis: Recurrent instances of inflammation and eosinophil activation may culminate in the accumulation of extracellular matrix proteins, leading to fibrosis of the gastrointestinal mucosa. This

fibrosis may contribute to the persistence of symptoms, including motility problems, seen in chronic FD.

3. Potential Transition to Other Disorders: Chronic eosinophilic inflammation in FD may predispose patients to other gastrointestinal disorders, including eosinophilic gastroenteritis or functional gastrointestinal disorders (FGIDs) with overlapping symptoms, such as irritable bowel syndrome (IBS). Comprehending these connections may yield insights on the enduring management of FD.
4. Systemic Effects of Chronic Inflammation: Chronic eosinophilic inflammation may exert systemic effects, potentially impacting other organs and contributing to comorbid diseases. The persistent inflammatory condition linked to eosinophil activation in FD may exacerbate systemic inflammation, potentially affecting other inflammatory disorders such as asthma, atopic dermatitis, or cardiovascular disease.
5. Immunological Dysregulation: Chronic eosinophil activation may result in extensive immunological dysregulation, thereby heightening the risk of autoimmune illnesses or worsening pre-existing problems.[39,41]

Molecular Therapeutics and Precision Medicine

As the comprehension of eosinophil participation in FD expands, various innovative therapy approaches are being investigated:

1. Biologics Targeting IL-5 and Eotaxin: Monoclonal antibodies such as mepolizumab and reslizumab, which inhibit IL-5, have demonstrated efficacy in decreasing eosinophil levels and alleviating inflammation in eosinophilic illnesses. Drugs that block eotaxin or its receptor, CCR3, may potentially diminish eosinophil migration to the gut in functional dyspepsia (FD).
2. Mast Cell Stabilizers: Due to the intimate relationship between eosinophils and mast cells, medications that stabilize mast cells and inhibit their degranulation may mitigate the subsequent effects of mast cell activation on eosinophils and ameliorate symptoms of FD.

Precision Medicine in FD

The diversity in FD symptoms among patients indicates that a universal treatment strategy may be ineffective. Progress in genetic and molecular profiling may facilitate precision medicine in FD.

1. Genetic Profiling: Identifying genetic variations in cytokine genes, such as those encoding IL-5 or IL-13, may facilitate the prediction of patients predisposed to eosinophil-driven FD. Patients may benefit from tailored medicines that particularly regulate eosinophil activity.

2. Biomarker Development: The creation of biomarkers indicative of eosinophil activity or associated immunological responses may facilitate the stratification of patients according to their underlying pathophysiology, resulting in more tailored treatment approaches.

3. Genetic Susceptibility: Genetic predisposition significantly influences an individual's vulnerability to FD, especially in instances involving eosinophils.

a) Cytokine Gene Polymorphisms: Variants in genes that encode cytokines, including IL-4, IL-5, and IL-13, may affect the extent of eosinophil activation and recruitment. Polymorphisms in the promoter region of the IL-5 gene may enhance IL-5 expression, leading to elevated eosinophil counts and an intensified inflammatory response. These genetic variations may elucidate why certain individuals with FD exhibit more prominent eosinophil-mediated inflammation and experience more severe symptoms.

b) HLA Associations: Certain human leukocyte antigen (HLA) alleles may be linked to a heightened risk of eosinophil-mediated disorders. HLA-DQ2 and HLA-DQ8 are related to eosinophilic esophagitis and may also contribute to functional dyspepsia, especially in eosinophil-rich subtypes.

c) Genetic Investigations and Prospective Research: Genome-wide association studies (GWAS) may be employed to find additional genetic markers associated with eosinophil activity and FD. This research may identify novel treatment targets and elucidate the genetic underpinnings of this illness.

Clinical studies on relationship between FD and Duodenal eosinophilia

Friesen et al(2004) did a double blind, randomized, placebo-controlled, cross-over study where they evaluated the effect of monteleukast in children with duodenal eosinophilia which they defined duodenal eosinophil count of more than 10 eosinophils/hpf. they enrolled forty children and adolescents with dyspepsia and duodenal eosinophilia between age 6 to 18 yrs. Subjects were randomized to receive either 10 mg once daily montelukast or placebo for 14 days and then assessed for symptomatic and biochemical responses. A statistically significant positive clinical response was observed in 62.1% of patients as per a post treatment global pain assessment receiving montelukast compared with 32.4% on placebo ($p < 0.02$). The Pain assessment scores were found worsened in five out of eleven responders (45%) after cross-over to placebo and improved in eight out of thirteen subjects (62%) of placebo non-responders on cross-over to montelukast. 84% of patients(nineteen) with a peak duodenal eosinophil counts between 20-29/hpf reported a positive pain assessment response in montelukast group compared to 42% receiving placebo ($p < 0.01$). No differences in response rates were observed at baseline. Dose or calculated drug exposure were not associated with the level of post treatment pain assessment or the change in biochemical markers based on pharmacokinetics assay. This study suggested a beneficial role for montelukast in the treatment of pediatric patients with FD associated with duodenal eosinophilia. [48]

Talley et al(2007) conducted a study with an aim to identify role of eosinophils in the upper gastrointestinal tract in non-ulcer dyspepsia in Swedish adults with a mean age of 54 years out of which 51% were females. A total of fifty one cases and forty eight controls were enrolled for the study after upper gastrointestinal endoscopy biopsies were taken from 5 sites (cardia, body, antrum, D1 duodenal bulb, and D2 second portion of duodenum) and reported by two independent observers. Median duodenal eosinophilia was taken as >/=22/hpf. The

odds ratio for non-ulcer dyspepsia vs asymptomatic controls in patients with high duodenal bulb and D2 eosinophil counts was 11.7 and 7.3 (95% confidence interval, 3.9-34.9 and 2.9-18.1) respectively after adjustments for H pylori, age and sex. Eosinophilic degranulation was found in seven cases. None of the controls had eosinophilic degranulation. Duodenal eosinophils (p-value<0.01) but not eosinophilic degranulation (p-value 0.11) or gastric eosinophil counts were found to be significantly increased in patients of non-ulcer dyspepsia. Early satiety was seen to be significantly associated with eosinophilia in D1 (P = .01) and D2 (P = .02) [49]

Talley et al(2008) published a paper addressing the heterogenous group of functional gastrointestinal disorders (FGIDs) that cause considerable public health issue in being remarkably common, disabling, resulting into a significant social and economic burden on health care facilities across the globe. They further emphasized the scarcity of understanding of the pathophysiology, diagnostic clinical criteria, laboratory or other biochemical markers for these conditions. The authors highlighted the unravelling weak evidence of association between duodenal eosinophilia and colonic mastocytosis among various other immune mechanisms contradictory to mere somatization to play a possible role in disease development and progression. Apart from this, these FGIDs have a considerable but unexplained overlap with a variety of other conditions which need to be evaluated in order to develop specific treatment targets in order to improve the quality of care and quality of life of these patients with FGIDs. The group also highlighted the need for better definitions, diagnostic criteria and severity assessment parameters for these diseases. The influence of various intestinal and extra-intestinal symptom and psychological distress on disease course and severity also requires in depth understanding. A better understanding of the pathophysiological aspects contributing to FGIDs, in turn, would not only help to identify newer and better treatment targets and approaches, but also reduce the health care burden cost in terms of reduction in number of

unnecessary investigations and interventions these patients undergo in routine clinical practice. [50]

Rothenberg et al(2008)discussed the same in their paper underscoring functional dyspepsia (FD), or non-ulcer dyspepsia, as a prevalent yet enigmatic gastrointestinal disorder, affecting as high as 25% of the population across the globe. It is characterized by persistent upper gastrointestinal symptoms without detectable abnormalities in endoscopic and histologic examinations. The pathogenesis of FD remains unclear, but recent studies suggest a link to abnormal motor and autonomic responses in the duodenum, potentially triggering pain and abnormal gastric emptying. A significant study by Talley et al. explored this connection by examining a large population in Sweden, where 1001 individuals underwent random upper endoscopy alongside completing validated questionnaires on abdominal symptoms. The researchers discovered an association between FD and increased levels of duodenal eosinophils—immune cells involved in inflammatory responses. After adjusting for factors like age, gender, and Helicobacter pylori infection, they observed not only higher eosinophil levels but also clustering and possible degranulation, indicating active inflammation. The study's findings suggest that changes in duodenal eosinophils, rather than gastric eosinophils, might be a key factor in FD's pathogenesis. However, these findings are preliminary and require replication, especially since previous research suggested that eosinophil activation could be a normal occurrence in the gastrointestinal tract. The study raises questions about the role of eosinophils in FD, given their involvement in other eosinophil-associated gastrointestinal diseases (EGID), such as eosinophilic esophagitis (EE). Eosinophils can cause tissue damage through the release of cytotoxic proteins and inflammatory mediators, potentially linking them to the symptoms of FD. The study encourages further research into the role of eosinophils and other inflammatory cells in FD and similar gastrointestinal disorders, with potential therapeutic

implications, including the use of anti-inflammatory agents and biologics currently in clinical trials. [51]

Walker et al(2008) also discussed about the increasing role of inflammatory pathway in pathogenesis of non ulcer dyspepsia. The eosinophil-mast cell-neural pathway was implicated to play an important in the pathophysiology of functional gastrointestinal disorders characterized by unexplained abdominal pain, disordered defecation, or meal-related discomfort. They emphasized the role of eosinophils in dyspepsia and mast cells in lower gastrointestinal symptoms supporting a role for a hypersensitivity-type reaction driving these disorders. Trigger could be food, pathogen, or other mucosal allergens which evoke eosinophils, mast cells, and other inflammatory markers in the cascade resulting in up-regulation of serotonin release, with modulation of the enteric and central nervous systems. This in turn creates a vicious cycle. They further suggested the potential role of targeting the eosinophil-mast cell pathway in treating these disorders.[52]

Friesen et al (2009) conducted another study in twenty-four dyspeptic patients aged 8-17 years out of which eighteen had elevated duodenal eosinophil density. These patients received Montelukast 10mg once daily for twenty one days followed by a repeat blood sampling and endoscopy. Global clinical response on a 5-point Lickert-type scale, eosinophil density utilizing H & E staining, eosinophil activation determined by degranulation indices on electron microscopy, and serum cytokine concentrations were assessed. Also, pharmacokinetics and duodenal mucosal drug concentrations on twenty one days were evaluated. The authors reported a positive clinical response to montelukast in eighty three percent subjects with regard to relief of pain. 50% of patients had a complete or nearly complete clinical response which was unrelated to mucosal drug concentration or systemic drug exposure. However, no significant changes in eosinophil density, eosinophil activation, or serum cytokine concentrations

following treatment with montelukast except a mild decrease in eosinophil density in the second portion of the duodenum. A negative correlation between pre-treatment TNF-alpha concentration with clinical response was seen. They concluded that the mechanism of action of Montelukast remains to be elucidated and does not appear to result from changes in eosinophil density or activation over the short term. [53]

Walker et al(2009) hypothesized that Mast cells and eosinophils might interact with T lymphocytes altering the enteric nerve and smooth muscle function in non organic dyspepsia. To prove this, mast cell, eosinophil and intraepithelial lymphocyte populations were examined in duodenal biopsies of subjects with IBS and FD in 51 cases each with FD and IBS. The findings were compared with 48 randomly selected controls with similar demographics. The authors showed that compared to controls, mast cells and IELs were significantly increased ($P < 0.001$ and $P = 0.005$, respectively) in IBS patients while functional dyspepsia patients had significantly increased eosinophils in D1 and D2 ($P < 0.001$). Therefore they attributed duodenal eosinophilia to FD and mast cell infiltration to IBS suggesting role of duodenal biopsy in differentiating these conditions. [34]

Sheikh et al(2009) Eosinophilic gastroenteritis (EG) is an uncommon disease characterized by focal or diffuse eosinophilic infiltration of the gastrointestinal tract, and is usually associated with dyspepsia, diarrhea and peripheral eosinophilia. Diffuse gastrointestinal tract and colonic involvement are uncommon. They reported cases of EG associated with gastric outlet and duodenal obstruction, acute pancreatitis, esophagitis, colitis and ascites with one eventually required surgery for recurrent gastric outlet obstruction. These cases highlight protean manifestations of EG involving any part of gastrointestinal tract like esophagitis, gastritis associated with ulcers, erosions or polypoidal lesions, pancreatitis, colitis and malignancy mimics.

Walker et al(2010) randomly selected 155 patients with a mean age 55 years, 59% being women with normal duodenal biopsies. All the records were analyzed for symptoms, diagnosis and medication and the patients were classified into five groups including a control group. The mean duodenal eosinophil count was 15/5HPFs in the control group. The prevalence of duodenal eosinophilia in the study group was 22.5%. The mean eosinophil counts (20.2/5HPF, $P < 0.04$) and prevalence of duodenal eosinophilia (47.3%, $P < 0.04$) were significantly higher in PDS subgroup. Duodenal eosinophilia was significantly associated with allergy (OR 5.04, 95% CI 2.12-11.95, $P < 0.001$) but no association was found with irritable bowel syndrome or medications. This study further elucidated a potential role of hypersensitivity mechanism in some patients with PDS including early satiety. [54]

Schurman et al(2010) conducted a pilot study to study effects of biofeedback-assisted relaxation training (BART) to medication treatment in twenty children with functional dyspepsia (FD) associated with duodenal eosinophilia. The subjects were randomly assigned to standard medication or medication along with 10 sessions of BART after a psychosocial functioning and quality of life measures at baseline, posttreatment, and 6 months. In this study, subjects receiving combination therapy demonstrated improved outcomes on intensity and duration of pain episodes alongwith clinical improvement. This study implies the need to recognize this newer entity as the treatment in this particular subset of patients differs from those without eosinophilia. [55]

In the study by Furuta, Liacouras, and Collins (2011) on eosinophilic esophagitis (EoE), the authors indirectly addressed role of duodenal eosinophilia within the broader context of eosinophilic gastrointestinal disorders. Duodenal infiltration by eosinophils was recognised as to have implication in functional gastrointestinal disorders including functional dyspepsia. Also to note that eosinophilic

esophagitis may have concurrent involvement of duodenum or other segments of the gastrointestinal tract contributing to symptom exaggeration. Therefore, understanding the role of duodenal eosinophilia contributes to the overall understanding of eosinophilic disorders and may have implications for diagnostic evaluation and management strategies. This study highlighted the need for comprehensive evaluation of eosinophilic inflammation throughout the gastrointestinal tract in patients with symptomatic diseases and underscores the potential for further research in the area. [56]

Binesh et al(2013) in a case control study compared eosinophil and mast cell populations in gastric and duodenal mucosal biopsies of adults with non-ulcer dyspepsia and non-dyspeptic adults in Iran. A total of 52 (25 cases and 27 control) patients were enrolled and gastric and duodenal biopsies were taken in all. The median eosinophil count in gastric mucosa in cases and controls was 5 and 4.0 respectively with a p value of 0.140. The same in duodenal mucosa was 16.0 and 13 respectively with a p value of 0.147. No significant difference in mast cells were found in either group. The frequency of Helicobacter pylori infection in both the groups was similar. Although a trend toward mild eosinophilia in gastric and duodenal mucosa was seen in non ulcer dyspepsia cases. [57]

Walker et al(2014) studied an Australian cohort of thirty three patients functional dyspepsia based on ROME II criteria and twenty two controls for upper gastrointestinal tract pathology, including duodenal eosinophilia. Duodenal eosinophilia in D2 was seen to be significantly increased in subjects with early satiety (P = 0.01) and postprandial fullness (P = 0.001). They did not find the same true for D1 biopsies. Abdominal pain was associated with eosinophilia in both first and second part of duodenum (P = 0.02 and 0.005). Smokers had and symptoms of early satiety (P = 0.02) and reported a higher eosinophil counts in second part of duodenum (P = 0.007). the authors thus implicated the potential role of duodenal eosinophils in FD. [58]

Wang et al(2015) recruited 141 FD patients and 39 healthy controls and evaluated duodenum (bulb and D2) for eosinophil counts with degranulation, and mast cell counts with degranulation. They reported significantly increased eosinophil counts along with higher proportion of eosinophilic degranulation at D2 in FD patients than healthy controls. Similar trends were seen in mast cell count and degranulation and it appears to be highly sensitive and specific for FD identification. The authors affirmed the potential role of these as histologic markers in FD.[59]

Du et al(2016) conducted a study in 96 patients with FD and 24 healthy controls. Although the eosinophil count was found to be comparable in the second portion of duodenum (D2) and duodenal bulb (D1) between patients with FD and healthy controls ($p > 0.05$), significantly higher rate of eosinophil degranulation was noted in D2 ($P = 0.003$) and D1 ($P = 0.084$) were observed in patients with FD. Furthermore, the number of duodenal degranulated eosinophil were significantly increased in patients with FD than healthy controls in D1(9.8 ± 6.3 vs 2.9 ± 2.1 per HPF, $P = 0.0002$) and a trend towards increase in D2 (10.7 ± 7.7 vs 5.3 ± 0.9 per HPF, $P = 0.077$), respectively implicating a role of same in pathogenesis of functional dyspepsia. No such difference was seen in mast cell degranulation in cases and controls. [60]

Wauters et al(2017) did a retrospective cohort study in children withn thirty-six cases and 36 nonmatched controls. 39% of enrolled patients had atopic history and 53% had psychological comorbidities which were significantly more frequent in cases than controls. Median duodenal eosinophil counts in cases was 151 (118-207) compared to controls 76 (60-106) per mm^2 ($P < 0.001$). An eosinophil count of >112 per mm^2 was found to be predictive for FD with an OR: 33.6 (95% CI: 7.1-159.0) and $p < 0.001$. also, patients with duodenal eosinophilia had higher odds of weight loss (OR: 7.1, 95% CI: 1.1-45.5; $P = 0.04$).The authors, therefore, argued a strong association of functional dyspepsia

with duodenal eosinophilia in pediatric patients illustrating multifactorial causation including atopic and psychological comorbidity. [61]

Chaudhary et al(2017) evaluated the histomorphological features of the duodenum and studied their relationship with *Helicobacter pylori* (*H Pylori*) infection in patients of FD in a case control study of 50 cases of FD patients and 30 age and sex matched controls. *H. pylori* was detected using rapid urease test on gastric antral biopsy. This study demonstrated that duodenal inflammation was invariably present in functional dyspepsia. Morphologically, increased IEL were identified in 72%, increased duodenal eosinophils in 92%, focal villous atrophy was present in 16%, lymphoid aggregates in 4%. 48% cases of FD were positive for gastric h pylori infection. Increased duodenal IEL count and duodenal eosinophilia was noted in 75% and 87.5% in h pylori positive cases and 61.5% and 95.15% cases with gastric *H. pylori* negativity, respectively. A statistically significant difference in duodenal IEL and eosinophil count in lamina propria was seen in functional dyspepsia patients compared with control and they had positive correlation with gastric H pylori infection. CD 8 expression was higher in duodenal IEL and lymphocytes in lamina propria as compared to CD4. [62]

Silva et al(2018) pointed out towards lack of a standard criteria and scarcity of data to define a normal baseline eosinophil count in gastrointestinal tract in pediatric subjects. They concluded that in healthy subjects, eosinophils were absent in esophagus. *The* mean eosinophilic density in stomach was higher in the deep compared to superficial lamina propria. Eosinophil counts were 18.1 ± 17.0, 14.4 ± 12.0, and $51.5 \pm 35.3/mm^2$ in the lamina propria of the bulb, D2, and ileum, respectively. In *Large intestine*, caecum had the highest peak count of 125 mm^2 with a mean of $51.8 \pm 33.5/mm^2$. Lower eosinophil counts were observed in the ascending (40.9 ± 27.4), transverse (34.3 ± 21.9), descending (40.0 ± 26.6), and sigmoid (25.8 ± 17.8) colon and in the rectum 13.9 ± 10.1. [63]

Sarkar et al(2019) did a prospective observational study comparing eosinophil count in duodenal mucosa between patients with functional dyspepsia and controls (n=42 each). They found a significantly increased duodenal eosinophil count was found in functional dyspepsia group than non dyspeptic patients (22.78±08.78 vs. 14.90±10.70) which was statistically significant with a p-value of 0.001. Eosinophil count was higher in subjects with postprandial distress syndrome. [64]

Lindiolo at al (2019) conducted a case control study showed that duodenal eosinophilia was found with significant difference in a subset of patients with early satiety (p=0.01). There was no difference in patients with postprandial fullness (p=0.63), epigastric pain or burning (p=0.26), gastroesophageal reflux symptoms (p=0.13), allergy and food intolerance (p=0.42) and smoking (p=0.28). They did not find a relationship between duodenal mastocytosis and early satiety (p=0.98), postprandial fullness (p=0.78), and epigastric pain or burning (p=0.82).[65]

Burns et al(2019) conducted a systematic review to look for evidence of immune activation in patients with functional dyspepsia and irritable bowel syndrome including about 51 articles and concluded that changes in lymphocyte populations, both B and T lymphocyte quantitatively as well as their activation status were reported in IBS and FD. Duodenal eosinophilia was seen in FD whereas colonic mast cells were found raised in IBS. There is some evidence to suggest a plausible role of Th17 responses despite paucity of immune phenotyping studies. Furthermore, circulating α4+β7+ gut-homing T cells also appear to be play a role in the pathophysiology of both these disorders by altering the mucosal homeostasis. But, most of the studies in the context have various limitations like poor phenotyping of patients into subgroups, small sample sizes, publication bias and heterogeneity. Although immune involvement is evident, large, well-characterized patient cohorts are required to elucidate the immune mechanisms driving the development of FGIDs. [66]

Ronkainen et al(2019) aimed To identify the association between duodenal eosinophilia in functional dyspepsia and symptoms of GERD and find out any association between symptoms of post-prandial distress syndrome or epigastric pain syndrome with new onset GERD. They enrolled 1000 participants from the national Swedish population register who were surveyed using questionnaires and esophagogastroduodenoscopy in 1999-2001. About 79% of the subsequently found 887 eligible subjects followed up in a study in 2010. Duodenal histology was evaluated in a case-control study of 213 subjects (functional dyspepsia vs healthy controls) from this population. The odds of GERD in patients with functional dyspepsia and post-prandial distress syndrome at baseline were OR 7.6(95% CI 2.93-19.4, P < 0.001) and OR 9.0 (95% CI 3.36-24.0, P < 0.001) respectively. Eosinophilia in the second part of duodenum only was independently associated with an increased risk of GERD amongst those with functional dyspepsia (OR 4.2; 95% CI 1.2-4.77, P = 0.024) and post-prandial distress syndrome at baseline (OR 6.0; 95% CI 1.50-23.6, P = 0.011), respectively. From this study, it can be safely concluded that duodenal eosinophilia is associated with increased risk of GERD over 10-year follow-up in subjects with baseline functional dyspepsia and post-prandial distress syndrome. This study also substantiates the unexplained overlap between functional dyspepsia and gastro-esophageal reflux disease (GERD) which has long been though to occur more often than expected by chance. One of the possible explanation may also be that post-prandial distress syndrome leads to impaired gastric fundic accommodation thereby increasing the transient lower esophageal sphincter relaxations and consequent GERD. This suggests that this subset of functional dyspepsia and GERD may be part of the same disease spectrum. [67]

Taki et al(2019) carried out a study to assess the role of inflammatory cell infiltration of the duodenum, H pylori infection, and genes associated with TJ proteins in duodenal biopsy specimens from 35 patients with FD and 31 asymptomatic controls. They counted the

immunohistochemically detected eosinophils and mast cells. The expression of claudins, occludin, and zonula occludens (ZO)-1 mRNA was evaluated using quantitative RT-PCR. Serum antibodies, rapid urease or urea breath tests, and endoscopic findings were studied to identify H pylori infection. They concluded that eosinophils and mast cells were significantly increased in patients with FD compared with controls and were significantly correlated. The inflammatory cells were not associated with H pylori infection status, gender or age. In addition, Claudin-3 mRNA expression was seen to be increased in the functional dyspepsia patients. This study further highlights the role of subtle duodenal inflammation with symptom onset and persistence. [35]

Halland et al(2019) compared the duodenal histopathological findings of patients with rumination disorder with healthy controls since these patients have a considerable overlap with functional dyspepsia**.** They recruited 22 patients with rumination syndrome with a mean age of 39.2 years (range 21-71) and 10 controls with a mean age of 34.3 (range 27-69). A significant increase in the mean eosinophil count and intraepithelial lymphocyte count was found among cases, 26 per mm^2 (range 16-42) and mean 15/100 enterocytes, range 8-29, as compared to controls 18 per mm^2 (range 10-28) and mean 11/100 enterocytes, range 11-18 with a p value of 0.006 and 0.02 respectively.[68]

Liete et al(2020) evaluated the role of Helicobacter pylori infection on eosinophilic infiltration in duodenal mucosa is poorly studied. Functional dyspepsia cases were included based on Rome III criteria and submitted to upper endoscopy. H. pylori infection was studies in gastric histopathology and urease test. The duodenal eosinophil count was found to be significantly higher in subjects positive for infection with median count of 13.2 compared to 8.1 in non-infected individuals (P=0.005). they, however, did not report a significant association between functional dyspepsia and duodenal eosinophil counts. [69]

Sarkar et al(2020) conducted a prospective observational study in Arab population of 84 subjects comprising of 42 cases of FD and 42 healthy control. The cut off of duodenal eosinophilia was defined as ≥22/5HPF.57.1% of patients with FD were reported to have duodenal eosinophilia**.** Duodenal eosinophil count was significantly higher in cases than patients controls (p = 0.001). A significant positive association was reported between duodenal eosinophilia and FD (OR = 5.67, 95% CI 1.92-17.2, p = 0.001)and postprandial distress syndrome (OR = 5.54, 95% CI 0.86-45.24, p = 0.036). A higher odds ratio was also noticed among those who complain of early satiety. [70]

Potter et al(2020) conducted a pilot randomized double-blind, placebo controlled study to evaluate the long hypothesized association between subset of the patients of functional dyspepsia with duodenal eosinophilia as possibly a consequence of environmental allergen. Since Non-coeliac gluten or wheat sensitivity (NCG/WS) has a considerable overlap of symptoms with these subjects, the authors studied the contributary role of wheat (specifically gluten and fructans) in these patients in a dietary crossover trial using modified salerno criteria. Patients fulfilling Rome III criteria FD were recruited from a single tertiary centre in Newcastle, Australia. Each of them were thoroughly counselled for a diet low in gluten and fermentable oligo-, di-, mono-saccharides, and polyols (FODMAPs) by a clinical dietitian. They were then subjected to a four weeks elimination diet phase. Those reporting a more than 30% response to the run-in diet, measured by the Nepean Dyspepsia Index, were subsequently re-challenged with either gluten, fructan, or placebo in randomized order. Reappearance of dyspepsia symptoms, i.e., a mean increase in overall dyspeptic symptoms of at least 30% on re-challenge were ascribed to have wheat induced FD. Out of eleven enrolled participants out of which 75% were female with a mean age of participants being 43 years, nine successfully completed the elimination diet phase. Of these, four eventually qualified for the rechallenge phase. The elimination diet led to a non-significant improvement in symptoms of

functional dyspepsia in the diet elimination phase with mean napean dyspepsia index symptom score of 71.2 vs. 47.1, p = 0.087. however, this study could not identify the specific food triggers. Small sample size remains a major limitation of this study to derive any conclusions.[71]

Nojkov et al(2020) also studies the role of duodenal epithelial barrier impairment and immune activation in the pathogenesis of functional dyspepsia (FD). They evaluated the duodenal epithelium for detectable microscopic structural abnormalities in 16 functional dyspepsia patients and 18 healthy controls in a first ever prospective study using confocal laser endomicroscopy (CLE) and mucosal biopsies. Epithelial gaps density or cell extrusion zone was measured considering it being a validated endoscopic measure to assess intestinal barrier status. Duodenal biopsies were evaluated for standard histology, quantification of mucosal immune cells/cytokines, immunohistochemistry for inflammatory epithelial cell death called pyroptosis and transepithelial electrical resistance (TEER) using ussing chambers. Epithelial cell-to-cell adhesion proteins expression was assessed by real-time polymerase chain reaction. Functional dyspepsia patients were noted to have significantly higher epithelial gap density on CLE in the distal duodenum compared to controls (P = 0.002), which corresponded to significant changes in the histopathology, impaired mucosal integrity by TEER (P = 0.009) and increased number of epithelial cells undergoing pyroptosis (P = 0.04). Reduced TEER was seen to have a inverse correlation with the severity of certain dyspeptic symptoms. Claudin-1 and interleukin-6 expression was altered in FD patients. This study re-emphasizes the role of epithelial barrier impairment and increased cell death in the pathogenesis factor in FD while also introducing CLE aa a potential diagnostic biomarker in FD. [72]

Ronkainen et al(2021) tried to further elucidate the potential role of duodenal eosinophilia with anxiety in functional dyspepsia by perturbing gut brain axis via microbial or inflammatory pathways in Swedish cohort. The enrolled subjects were made to fill a validated

Abdominal Symptom Questionnaire, the Hospital Anxiety and Depression Scale questionnaire. Duodenal biopsies from first(D1) and second portion of duodenum(D2) were taken. 887 enrolled subjects were invited to participate in a 10-year follow-up study with the same questionnaires. The final study population comprised 89 cases with FD and 124 healthy controls (mean age 62 years, SD 12, 34% male). Patients with D1 eosinophilia at baseline were found to have clinically elevated anxiety levels with an odds ratio for new-onset anxiety and or follow-up anxiety adjusting for baseline anxiety 4.51 (95% CI 0.8, 23.8; p = 0.08) and 4.5 (95% CI 1.03, 19.81; p = 0.046) respectively, thereby implicating a role of duodenal eosinophilia in anxiety related symptoms[73]

Chehade et al(2021) did a observational cohort study in order to characterize the diagnosis of eosinophilic gastritis or eosinophilic duodenitis to overcome the lack of a standardized definition. They reported a mean delay of 3.6 years in diagnosis from onset of symptoms attributable to delayed gastroenterologist referral, delayed esophagogastroduodenoscopy (EGD), and lack of biopsy collection and/or histopathologic evaluation. Approximately 38.2% of patients were missed on index biopsy resulting in a further delay of 1.6 years. This study demonstrated adult age; prior diagnosis of irritable bowel syndrome, functional dyspepsia, or gastric/peptic ulcer; use of other procedures such as colonoscopy; presence of edema; and history of certain allergic diseases as independent predictors of diagnostic delay. This highlights the increased need to heightened awareness and standardization of diagnostic guidelines for the disorder. [74]

Wauters et al(2021) evaluated the effect of proton pump inhibitors in duodenal and systemic alterations in FD patients and healthy volunteers (HVs) in a prospective interventional study. Symptoms based on Patient Assessment of Gastrointestinal Symptom Severity Index, duodenal alterations, and systemic factors in patients with FD ("FD-starters") and HVs before and after PPI therapy over four weeks

alongwith duodenal eosinophils, mast cells, permeability, luminal pH and bile salts were in duodenal aspirates were assessed. A total of 30 HV, 27 FD-starters, and 18 FD-stoppers(defined as non responders at 8 weeks) completed the study. Symptoms and duodenal eosinophils, mast cells (all, P < .0001), and paracellular passage (P = .02) were found to be significantly higher in FD-starters vs HVs. Dyspeptic symptoms significantly correlated with eosinophils before and during PPI therapy and the same decreased following therapy. FD-stoppers also reported a reduction in symptoms as well as immune cells off PPIs. In contrast, immune cells and permeability increased in HVs on PPIs and this was also associated with changes in bile salts concentration in duodenal aspirates. [75]

Dellon et al(2021) conducted a study and proposed that in patients with moderate-to-severe GI symptoms, eosinophilia in gastric or duodenal biopsies tends to be patchy in distribution. Therefore, they endorsed acquiring a minimum of four biopsies from duodenum and eight from gastric mucosa. [76]

Shah et al(2022) did a systematic review and meta-analysis to evaluate the relation between duodenal eosinophils and mast cells in patients with functional dyspepsia including twenty two case control studies with 1108 patients and 893 controls done till June 2021. Duodenal eosinophils and mast cells were significantly higher in functional dyspepsia patients compared to controls with a standardized mean difference of 1.29 and 2.11 (95% CI, 0.85-1.73; P = .0001 and 95% CI, 1.14-3.07; P = .0001) respectively. Degranulation of duodenal eosinophils was significantly higher in FD patients compared with controls with an odds ratio of 3.78 (95% CI, 6.76-4.48; P = .0001), without statistically significant heterogeneity. Postinfectious FD patients and helicobacter pylori negative patients had significantly increased duodenal eosinophils compared with controls. No significant difference was seen in functional dyspepsia subtypes in this analysis. Thus, a possible link between duodenal eosinophilia and functional dyspepsia

could be derived from this study but in presence of a low quality of evidence owing to substantial heterogeneity and publication bias seen in various studies, a confirmation for the same requires further evaluation. [77]

A case-control study of 150 consecutive subjects consisting of 100 patients with functional dyspepsia and 50 asymptomatic healthy controls evaluated duodenal histology for the number of eosinophils, mast cells, and Helicobacter pylori presence. They reported a statistically significant difference in mast cell count but not duodenal eosinophils between people with functional dyspepsia and people without it. [78]

Min et al(2022) studied a Symptom-based subtyping of functional dyspepsia with similar baseline variables and their association with the duodenal and gastric microinflammation in twenty one FD patients. Eosinophil infiltration was seen to be more prominent in the duodenum compared to stomach but mast cell infiltration was similar at both sites. No difference in eosinophil counts was in the duodenum in the three subgroups, i.e, epigastric pain, postprandial distress and overlap groups. Similarly, no statistically significant difference was seen in the eosinophil counts in the stomach and mast cell counts in the duodenum and stomach. [79]

Another cross-sectional study done in 436 adults from ethnically diverse background defined duodenal eosinophilia cut-off as ≥ 63 per 5 HPF which was present in 50.5% or recruited cases. Eosinophil degranulation was seen in 23.1%. FD was observed in 178 patients (41.7%). They concluded that neither the mean eosinophil count nor duodenal eosinophilia was associated with FD, but eosinophil degranulation, especially early satiety was found to be independently associated with FD with an odds ratio of 1.74 and p-value of 0.02. [80]

Mahendra Raj et al(2022) evaluated the eosinophil counts in archived endoscopic duodenal biopsies and clinical charts of 289 subjects. On

logistic regression analyses, eosinophil counts were found to be positively associated with symptomatic erosive GERD (Odds Ratio, OR 1.03, 95% Confidence Interval, 95%CI: 1.00, 1.05; p=0.035) but not functional dyspepsia. Increased duodenal eosinophils were reported to be associated with symptomatic erosive gastro-esophageal reflux disease (OR 3.36, 95%CI 1.18,-9.60; p=0.023), the presence of co-morbidities (OR 2.00, 95%CI 1.10, 3.62; p=0.022), and Chinese but not Malay or Indian ethnicity. However, no association was seen between IBS, functional dyspepsia, h pylori infection, gender or gallstone disease. [81]

Barreyro et al(2023) conducted a multicenter prospective study comprising of a total of 516 patients who met Rome-III criteria were selected and normal endoscopy. In this study, 231 (45%) patients had epigastric pain syndrome (EPS), 168 (33%) postprandial distress syndrome (PDS), and 117 (22%) EPS/PDS overlap. Two hundred fifty-nine (50.3%) patients were found to be positive for H. pylori. No histological difference between FD subtypes was seen. Significantly increased in duodenal eosinophil densities (>10 per high power field) was seen in PDS compared with EPS and EPS/PDS overlap subtypes. The odds ratio of postprandial distress in patients with significant duodenal eosinophilia was 2.28 (95% CI, 1.66-3.14; P <0.0001 which further increased to OR 3.6 (95% CI, 2.45-5.28; P <0.0001) after adjusting for age, gender, H. pylori and nonsteroidal anti-inflammatory drug use. ROC curve analysis further demonstrated that in H. pylori negative subjects, low-grade duodenal eosinophilia was highly accurate for PDS with the AUC of 0.731 compared with H. pylori positive AUC of 0.598 in addition, duodenal eosinophilia was also correlated significantly with treatment response for 4 to 6 weeks of proton pump inhibitor therapy. Low grade duodenal eosinophilia was significantly associated with PDS subtype non- H. pylori FD patients and could be used as a objective marker for treatment response. [82]

Barreyro et al(2023) studied the impact of duodenal eosinophilia and host genetic polymorphism of innate and pro-inflammatory cascade

using NOD1-796G>A and IL-1B-511C>T gene variants in Helicobacter pylori infected individuals A multicenter cross-sectional study was conducted with 253 patients who met Rome-IV criteria of which 98 patients were included after unremarkable upper endoscopy and positive H. pylori in gastric biopsies. Clinical parameters, H. pylori cagA and duodenal histology, were evaluated. No association was found between FD subtypes and studied gene variants. Low-grade duodenal eosinophilia was significantly increased in NOD1-796 GG versus single A-allele, but not in IL-1B-511 single T-allele or CC-allele. A synergistic effect on low-grade duodenal eosinophilia was found between these two loci irrespective of cagA strain status in Hp. They concluded that low-grade duodenal eosinophilia is significantly associated with NOD1-796 GG allele specially in cagA strain and with allelic combination NOD1-796 GG/IL-1B-511 single T-allele independent of cagA strain infection in Hp patients. [83]

Barreyro et al(2023), in another study, explored the association of H. pylori virulence genes and low-grade duodenal eosinophilia in Helicobacter pylori infection in a multi-center cross-sectional study enrolling 301 patients using Rome-III criteria. 95 patients were included and evaluated for clinical parameters and duodenal biopsy. Further, they were assessed for H. pylori virulence genes (cagA, oipA, and vacA). In this study, 72% subjects had EPS, 18%PDS and 10%EPS/PDS overlap. FD syndromes were not found to be associated with cagA or oipA strains. EPS had a significant trend with vacA s1/m1 (78%) and s1/m2 (80%) positive strains. Low-grade duodenal eosinophilia was seen significantly in cagA positive strain with odds ratio of 4.2 (95% CI, 1.78-9.93) or 5.44(1.989-14.902), after adjusting for age, gender, smoking, diabetes, alcohol, PPI, and NSAID, but no difference was seen in oipA and vacA strains. [84]

Huang et al(2023) conducted a comprehensive review of literature comprising 338 studies in FD and anxiety/depression from 2003 to 2023. Studies related to FD and anxiety/depression have demonstrated

an ascending trajectory since 2003. The timeline view map or trend-term analysis suggested that duodenal low-grade inflammation ("duodenal eosinophilia" and "mast cells") might be a new concern associated with FD and anxiety/depression. [85]

Dellon et al(2024) conducted a pooled analysis comprising four prospective studies to ascertain eosinophil thresholds in gastric and duodenal mucosa. Based on the highest specificity analysis in 740 patients, the optimal eosinophil threshold was determined to be 20 eos/HPF in 5 gastric HPFs for Eosinophilic gastritis (71% sensitivity and 94% specificity) and 33 eos/HPF in 3 duodenal HPFs for Eosinophilic duodenitis (49% sensitivity and 100% specificity). For single-field analysis, the optimal eosinophil thresholds were 33 eos/HPF (Eosinophilic Gastritis) and 37 eos/HPF (Eosinophilic Duodenitis), both corresponding to 93% sensitivity and 93% specificity. These highly specific single gastric and duodenal HPF thresholds may have more clinical applicability than thresholds requiring multiple HPFs and could better facilitate development of practical histopathologic guidelines to aid pathologists and clinicians in the detection and diagnosis of these. [86]

Ceulemans et al(2024)aimed to standardize the histological criteria for duodenal eosinophilia. Software-based histological cell quantification was developed and applied to digitized hematoxylin and eosin (H&E)-stained slides from 58 individuals HVs and patients with functional dyspepsia. Intraclass correlation coefficients (ICCs) were employed to compare the inter-rater reliability between software-based and microscopic quantification methods. Reproducibility was validated. H&E based eosinophil identification was further compared to immunohistochemistry (IHC). Normal range for eosinophil based on H&E staining and mast cell based on cKit were determined in 70 adult HV. Digitized slides demonstrated excellent (ICC = 0.909) and significantly improved reproducibility over microscopic evaluation (ICC = 0.796, P = 0.0014), which were subsequently validated. Duodenal

eosinophils were seen to be more abundant around the crypts than villi (P< 0.0001), while counts were similar on matched H&E- and IHC-stained slides (P = 0.55). Mean ± SD (95th percentile) duodenal eosinophils and mast cells in HC were 228.8/mm^2 ± 94.7 (402.8/mm^2) and 419.5/mm^2 ± 132.2 (707.6/mm^2), respectively. The software-based quantification so developed, identified 400 eosinophils/mm^2 and 700 mast cells/mm^2 as thresholds for abnormal duodenal infiltration. [87]

Irani et al(2024) performed a systematic review and meta-analysis of thirty one papers to ascertain the association between CD and GERD. CD was defined based on serological and histological parameters. GERD was defined by classical symptoms, endoscopic or histologic esophagitis or 24-h pH study. The study suggested that celiac disease patients on gluten containing diet were thrice as likely to have GERD compared to controls (OR: 3.37, 95% CI: 2.09-5.44). The risk increased to over 10 times when compared to those on a gluten free diet (GFD) (OR: 10.20, 95% CI: 6.49-16.04). The study also demonstrated an increased risk of endoscopic esophagitis with CD (OR: 4.96; 95% CI: 2.22-11.06). The authors also reported concluded that one year of a GFD in CD and GERD was more efficacious in preventing GERD symptom relapse as compared to 8 weeks of PPI in non-CD GERD patients (OR: 0.18, 95% CI: 0.08-0.36). furthermore, pediatric celiac disease patients were more likely to develop GERD symptoms compared to adults. This review further stressed on the role for duodenal inflammation and dietary antigens in the aetiology of a subset with GERD. [88]

Friesen et al(2024) evaluated the effect of ketotifen, a second-generation antihistamine and mast cell stabilizer, in a pilot study in these patients in a double blind, placebo controlled cross-over design pilot study in eleven patients over a 4 week period. Global clinical response was graded on a 5-point Likert Scale. A single plasma sample was obtained at steady state for pharmacokinetic analysis where half life was confirmed to be 3.3 hours. Complete or near-

complete clinical response was demonstrated in only 18% patients, implying the drug was not superior to placebo. Recommendations are being made for larger treatment trial however. [89]

Dalal et al(2024) explored the metabolic differences between early satiety subgroup of functional dyspepsia patients with duodenal mucosal eosinophilia to those without it enrolling an existing biorepository. Plasma samples were obtained at the same time as endoscopy. Patients were divided into two wo groups, those with peak duodenal mucosal eosinophil densities above 30/high power field (N = 28) and those below 30 (N = 16). The fasting plasma samples were assessed by liquid chromatography/high-resolution mass spectrometry. Significant elevations were demonstrated in several gamma-glutamyl amino acids, oxidative stress associated metabolites like glutathione metabolites (cysteinlyglycine and cys-gly oxidized), nitric oxide synthesis (arginine, citrulline, ornithine, and dimethylarginine), elevated vanillymandelate levels and alterations in lipid metabolism including several long-chain acylcarnitine conjugated fatty acids in the eosinophilia group. In contrast, carnitine levels were lower in the eosinophilia group. This study underscores the potential implication of increased oxidative stress and alterations in lipid metabolism in subsets of dyspepsia patients. which might explain the differences in therapeutic responses to various existing treatments and emphasize the need for newer developments. [90]

Variability among Patients and Eosinophil Function: Phenotypic variability in functional dysplasia

There exists considerable variety in the manifestation of FD, potentially because of variations in eosinophil activity among patients. This heterogeneity can affect the severity and nature of symptoms encountered, along with the therapeutic response. Patients with FD can be classified into subgroups, including epigastric pain syndrome (EPS) and postprandial distress syndrome (PDS), each potentially exhibiting distinct underlying pathophysiological mechanisms involving

eosinophils. Eosinophil-mediated inflammation may be more pronounced in PDS because symptoms are strongly associated with food consumption. Elevated eosinophil counts and clinical implications: Research indicates that patients exhibiting increased eosinophil counts in the duodenal mucosa generally experience more severe symptoms and demonstrate a worse response to conventional therapies. Utilizing endoscopic biopsy and other diagnostic modalities to identify these patients could facilitate the customization of treatment regimens to meet their specific requirements.

Customized Therapeutic Strategies

Due to the variability in eosinophil activity among FD patients, individualized treatment strategies are crucial.

Biomarker-Guided Therapy: The advancement of biomarkers indicative of eosinophil activity, including blood eosinophil counts or serum concentrations of eosinophil-associated cytokines, may assist in informing treatment choices. Patients exhibiting elevated eosinophil activity may get advantages from medicines aimed at eosinophils or their mediators, whereas individuals with diminished activity may require other management approaches.

Patient Stratification: Categorizing patients according to their eosinophil activity and additional immunological indicators may provide more effective, personalized treatment strategies, enhancing outcomes and alleviating the burden of FD.

The enhanced discourse now encompasses a thorough examination of eosinophil interactions with various immune cells, the prospects for innovative molecular therapies, the influence of genetic predispositions, and the enduring consequences of chronic eosinophil-mediated inflammation. Moreover, lifestyle and environmental factors, together with the heterogeneity among functional dyspepsia patients, have been

taken into account to offer a thorough explanation of the involvement of eosinophils in this condition.

This dissertation elucidates the role of eosinophils in the pathophysiology of FD through many processes, while proposing avenues for future research and potential treatment interventions. The emphasis on precision medicine highlights the necessity of customizing therapy for each patient, informed by their specific immunological and genetic characteristics

MATERIAL AND METHODS

The study is an observational cross sectional comparative hospital based study and will be conducted in Department of Medical Gastroenterology, Dayanand Medical College and Hospital, Ludhiana in collaboration with department of Pathology, Dayanand Medical College and Hospital, Ludhiana, Punjab. DMCH is a more than 1600 bedded tertiary care hospital in north Indian state of Punjab. An approval to conduct the study was obtained from institutional ethical review board. The study did not involve any diagnostic or therapeutic manoeuvre outside the routine evaluation of these patients and did not entail any extra costs to them.

Study population: Patients coming to the department for esophagogastroduodenoscopy were included in the study. Total numbers of cases were 150 including both males and females comprising of patients who presented with functional dyspepsia. They were compared with 50 age and sex matched control subjects from general population of same socioeconomic status who underwent endoscopy without symptoms of dyspepsia. Study was conducted after approval from the institutions ethics committee.

Informed Consent was taken from all the participants included in the study.
Group A: comprising of 150 people with functional dyspepsia
Group B: comprising of 50 age and sex matched control subjects.

The patients and controls in two groups (A&B) were subjected to routine history taking, systemic examination, esophago-gastro-duodenoscopy and duodenal biopsy. All patients had received deworming prior to taking duodenal biopsies within last one to six months as part of routine deworming policy of the institution. Detailed history was taken and SFNDI QOL questionnaire was filled by the

cases. The symptoms were later assessed in terms of frequency and severity and score was given from 0 to 9 for symptoms of EPS, PDS, retrosternal discomfort, bloating. Individual frequency and severity scores of EPS and PDS symptoms were combined to calculate score for overlap. Severity Score of 4 out of 9 was taken as significant symptoms. Individual symptom score of 4 or more in the two subgroups, i.e. EPS and PDS each were taken as overlap syndrome. The symptom score for overlap, thus, was calculated out of a total of 18. All the required laboratory investigations were noted.

INCLUSION CRITERIA (CASES)

- Age > 18 years.
- Functional dyspepsia meeting ROME IV criteria

  According to the Rome IV criteria, functional dyspepsia is defined by:

  Presence of one out of following symptoms : bothersome postprandial fullness or early satiety, borthersome epigastric pain or burning.

  Persistent or recurring dyspepsia for more than 3 months within the past 6 months

  No demonstration of a possible organic cause of the symptoms on endoscopy

  No sign that the dyspepsia is relieved only by defecation or of an association with stool irregularities.
- Normal esophago-gastro-duodenoscopy
- Normal stool microscopy

INCLUSION CRITERIA (CONTROLS)

- Age >18years.
- Subjects undergoing endoscopy for indications other than dyspepsia like anemia or diarrhea
- No history of active GI bleed.

EXCLUSION CRITERIA

- Positive rapid urease test
- Inflammatory Bowel Disease
- History of peptic ulcer
- Any eosinophilic condition of any other area of GIT, Parasitic infections as confirmed on stool microscopy for ova and cyst or Peripheral eosinophilia
- Any duodenal polyp, nodule, stricture or duodenitis
- Progressive severe disease requiring active medical management (CAD/ CKD/ malignancy/cirrhosis/uncontrolled diabetes or hypertension)
- History of upper gastrointestinal surgery
- Drugs causing eosinophilia like antiepileptics (carbamazepine, phenytoin, lamotrigine), fluoxetine, captopril and antibiotics (ampicillin, isoniazid, rifampin, linezolid, trimethoprim-sulfmethoxazole, vancomycin)
- Drugs causing duodenal inflammation like NSAIDS, aspirin or clopidogrel.
- Active alcohol, smoking or any other addiction

Statistical analysis

The presentation of the Categorical variables was done in the form of number and percentage (%). On the other hand, the quantitative data were presented as the means ± SD and as median with 25th and 75th percentiles (interquartile range). The data normality was checked by using Shapiro-Wilk test. The cases in which the data was not normal, we used non parametric tests. The following statistical tests were applied for the results:

1. The comparison of the variables which were quantitative and not normally distributed in nature were analyzed using Mann-Whitney Test (for two groups) and Kruskal Wallis test (for more than two groups) and variables which were quantitative and normally distributed in nature were analyzed using Independent t test.

2. The comparison of the variables which were qualitative in nature were analyzed using Chi-Square test. If any cell had an expected value of less than 5 then Fisher's exact test was used.

3. Univariate and multivariate logistic regression was used to find out significant risk factors of raised duodenal eosinophil in cases.

4. Receiver operating characteristic curve was used to assess cut off point, sensitivity, specificity, positive predictive value and negative predictive value of duodenal eosinophil count for predicting dyspepsia.

5. Spearman rank correlation coefficient was used to assess correlation of Duodenal eosinophil count with various parameters, for correlation of gastric eosinophils and age with various parameters.

The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, ver 25.0. For statistical significance, p value of less than 0.05 was considered statistically significant.

OBSERVATION AND RESULTS

RESULTS AND OBSERVATIONS

In this study, we analysed data of 150 patients of functional dyspepsia diagnosed as per ROME IV consensus who fulfilled the inclusion criteria from 1s^{t} January 2023 to 31st August 2024 and 50 age and sex matched controls.

The patients and controls in two groups (A&B) were subjected to routine history taking, systemic examination, esophago-gastro-duodenoscopy and duodenal biopsy. All patients received deworming prior to taking duodenal biopsies within last one to six months as part of routine deworming policy of the institution. Detailed history was taken and SFNDI QOL questionnaire was filled by the cases. The symptoms were later assessed in terms of frequency and severity and score was given from 0 to 9 for symptoms of EPS, PDS, retrosternal discomfort and bloating. Individual frequency and severity scores of EPS and PDS symptoms were combined to calculate score for overlap. Individual symptom score of 4 or more in the two subgroups, i.e. EPS and PDS each were taken as overlap syndrome. The symptom score for overlap, thus, was calculated out of a total of 18. SFNDI QOL quality of life score (SFNDI QOL QOL) was also calculated with maximum score of 50. All the required laboratory investigations were noted.

A cut-off for normal duodenal eosinophil count of 12/hpf was derived in healthy control population using receiver operator characteristic curve. Using this cut-off value of 12/hpf, the cases were divided into two groups: cases with normal duodenal eosinophil count (comprising of 53 subjects) and those with raised duodenal eosinophil count (comprising 97 subjects). The study included 150 patients of functional dyspepsia, with a diverse age distribution as shown in table no 2. The majority of patients were between 21 to 40 years (45.33%) followed by those between 41 to 60 years old (24%), above 60 years (19.33%) and below

20 years(11.33%). All the cases and controls were subjected to a duodenal biopsy and eosinophil count per high power field was seen.

Table 1:- Receiver operating characteristic curve of normal duodenal eosinophil count for healthy population.

Variables	Values
Area under the ROC curve (AUC)	0.749
Standard Error	0.0378
95% Confidence interval	0.675 to 0.823
P value	<0.0001
Cut off	<12
Sensitivity(95% CI)	64.67%(56.5 - 72.3%)
Specificity(95% CI)	84%(70.9 - 92.8%)
PPV(95% CI)	92.4%(85.5 - 96.7%)
NPV(95% CI)	44.2%(34.0 - 54.8%)
Diagnostic accuracy	69.50%

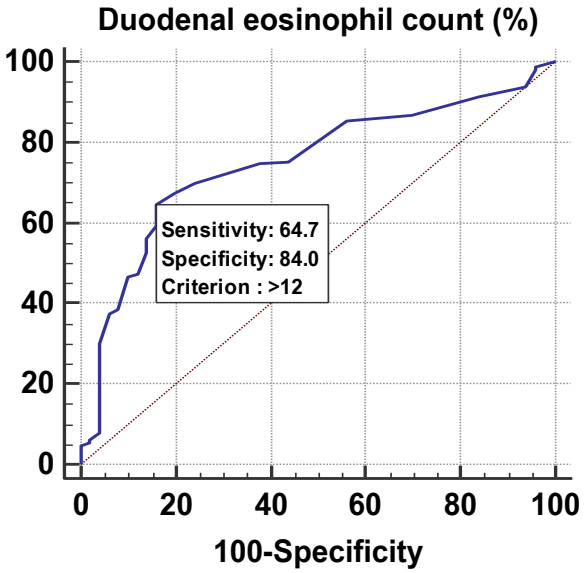

Figure 1:- Receiver operating characteristic curve of normal duodenal eosinophil count for healthy population

Cut-off of normal duodenal eosinophil count in healthy population was defined as 12/hpf with AUC 0.749; 95% CI: 0.675 to 0.823. (Table 1, figure 1).

Table 2:-Comparison of duodenal eosinophil (/hpf) between cases and controls.

Duodenal eosinophil (%)	Cases(n=150)	Controls(n=50)	Total	P value
Mean ± SD	18.08 ± 11.09	8.6 ± 7.66	15.71 ± 11.11	<.0001§
Median(25th-75th percentile)	20 (7.25-26)	6 (4-8)	14 (6-24)	
Range	0-60	0-35	0-60	

§ **Mann Whitney test**

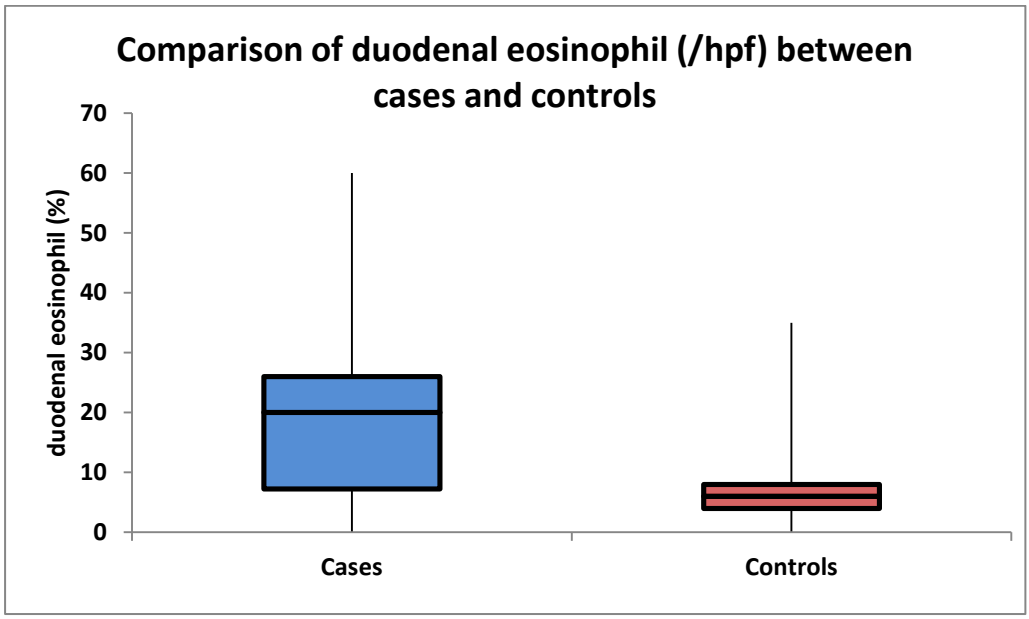

Figure 2:-Comparison of duodenal eosinophil (/hpf) between cases and controls.(non-parametric variable, Box-whisker plot)

Median (25th-75th percentile) of duodenal eosinophil percentage in cases was 20 (7.25-26)/hpf which was significantly higher compared to controls (6 (4-8)/hpf (p value < 0.0001)) (Table 2, Figure 2).

Table 3 :-Age Distribution of Cases

Age	Normal Duodenal eosinophil count(n=53)	Raised Duodenal eosinophil count (n=97)	Total (n=150)	P value
<=20 years	7 (13.21%)	10 (10.31%)	17 (11.33%)	0.569†
21 to 40 years	20 (37.74%)	48 (49.48%)	68 (45.33%)	
41 to 60 years	15 (28.30%)	21 (21.65%)	36 (24%)	
>60 years	11 (20.75%)	18 (18.56%)	29 (19.33%)	
Mean ± SD	42.79 ± 20.04	40.88 ± 16.55	41.55 ± 17.82	0.531‡
Median(25th-75th percentile)	40 (27-51)	40 (27-55)	40 (27-54.75)	
Range	8-98	11-76	8-98	

‡ Independent t test, † Chi square test

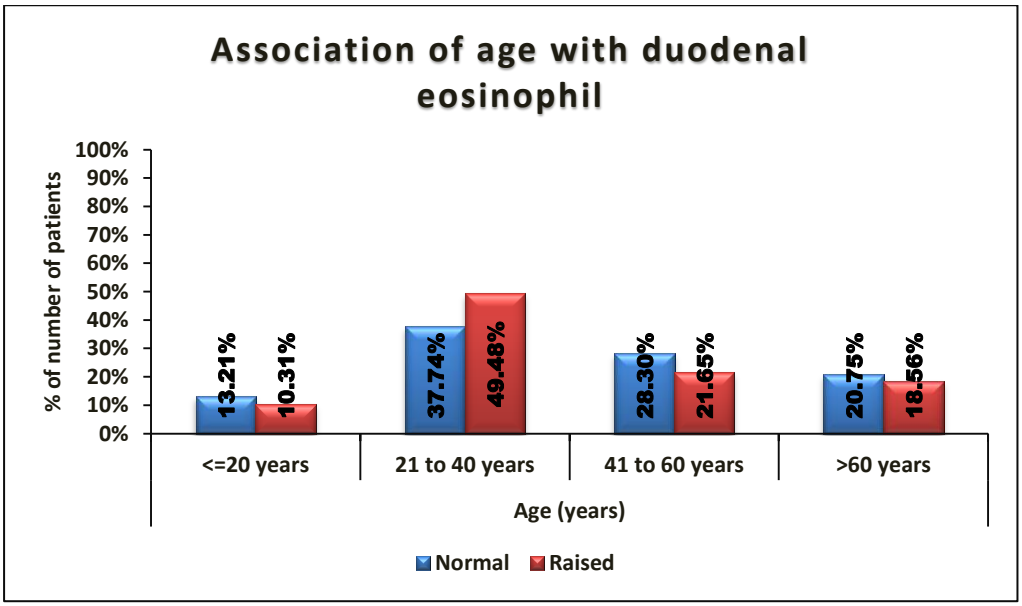

Figure 3 :-Age Distribution of Cases

Compared to normal duodenal eosinophil group, raised duodenal eosinophil group had comparable distribution of age: ≤20 years (Raised: 10.31% vs. Normal: 13.21%), 21 to 40 years (49.48% vs. 37.74%), 41 to 60 years (21.65% vs. 28.30%) and >60 years (18.56% vs. 20.75%) (p value = 0.569). Mean ± SD of age in raised duodenal eosinophil group was 40.88 ± 16.55 years, while in normal duodenal eosinophil group, it was 42.79 ± 20.04 years, with no significant association between them (p value = 0.531) (Table 3, Figure 3).

Table 4 :-Association of duodenal eosinophil count (/hpf) with age.

Duodenal eosinophil count (%)	<=20 years (n=17)	21 to 40 years (n=68)	41 to 60 years (n=36)	>60 years (n=29)	Total	P value
Mean ± SD	18.35 ± 12.66	18.88 ± 11.25	17.36 ± 10.39	16.93 ± 11.05	18.08 ± 11.09	0.906[¶]

[¶]**Kruskal Wallis test**

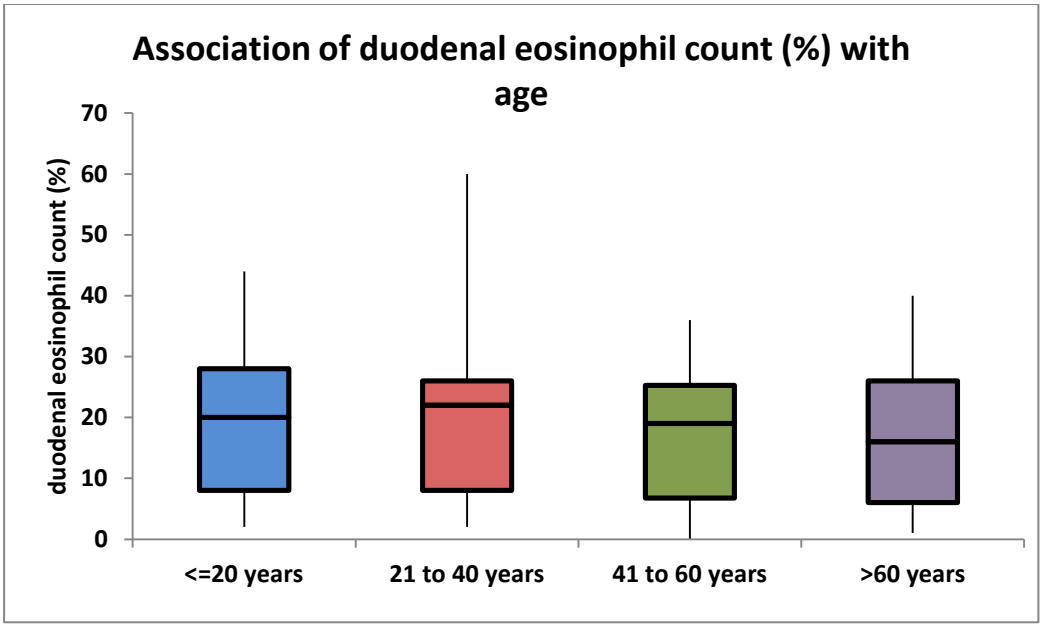

Figure 4:-Association of duodenal eosinophil count (/hpf) with age.(non-parametric variable, Box-whisker plot)

Mean with SD of duodenal eosinophil count was as follows: ≤20 years: 18.35 ± 12.66/hpf, 21 to 40 years: 18.88 ± 11.25/hpf, 41 to 60 years: 17.36 ± 10.39/hpf and >60 years: 16.93 ± 11.05/HPF, with no significant association between them (p value = 0.906) (Table 4, Figure 4)

Table 5 :- Descriptive statistics of dyspepsia subgroups symptom score(SS) in cases.

Dyspepsia subgroups Symptom Score	Mean ± SD
EPS SS (n=52)	7.4 ± 0.95
PDS SS(n=45)	6.97 ± 1.27
Overlap SS(n=53)	12.69 ± 1.51
Bloating SS	5.77 ± 1.67
Retrosternal discomfort SS	5.6 ± 2.08
SFNDI QOL QOL	28.73 ± 8.36
Duration of dyspepsia (years)	4.27 ± 3.4
PPI duration (years)	3.61 ± 2.6
Gastric eosinophils (%)	7.75 ± 3.62

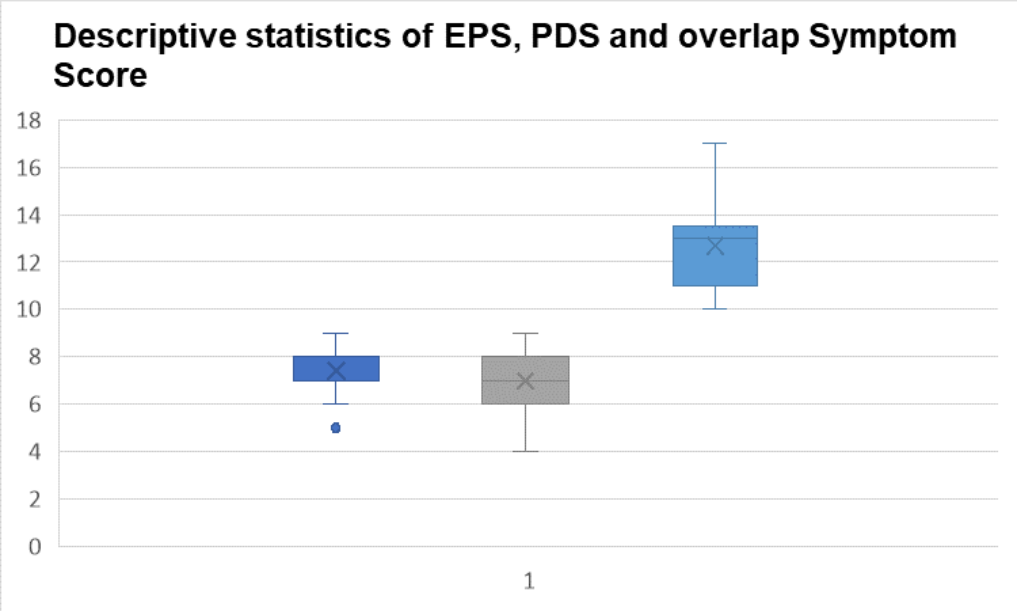

Figure 5.1:-Descriptive statistics of EPS, PDS and overlap Symptom Score.

Mean values of EPS, PDS and overlap symptom scores were 7.4 ± 0.95, 6.97 ± 1.27, 12.69 ± 1.51, 2.77 ± 1.67, respectively (Table 5, Figure 5.1).

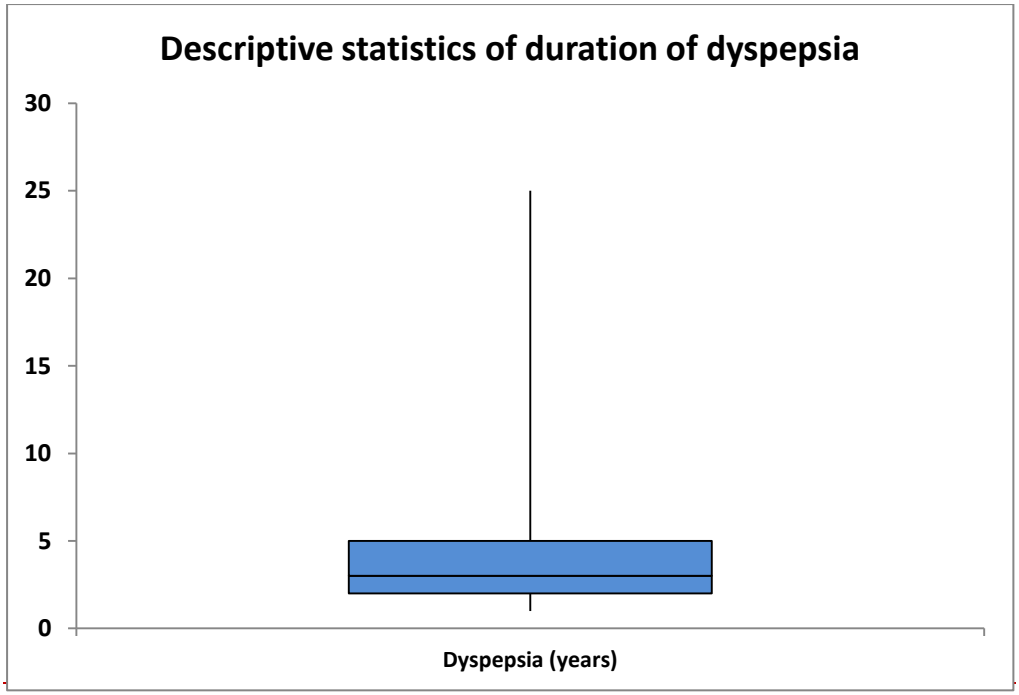

Figure 5.2 :-Descriptive statistics of duration of dyspepsia (years).

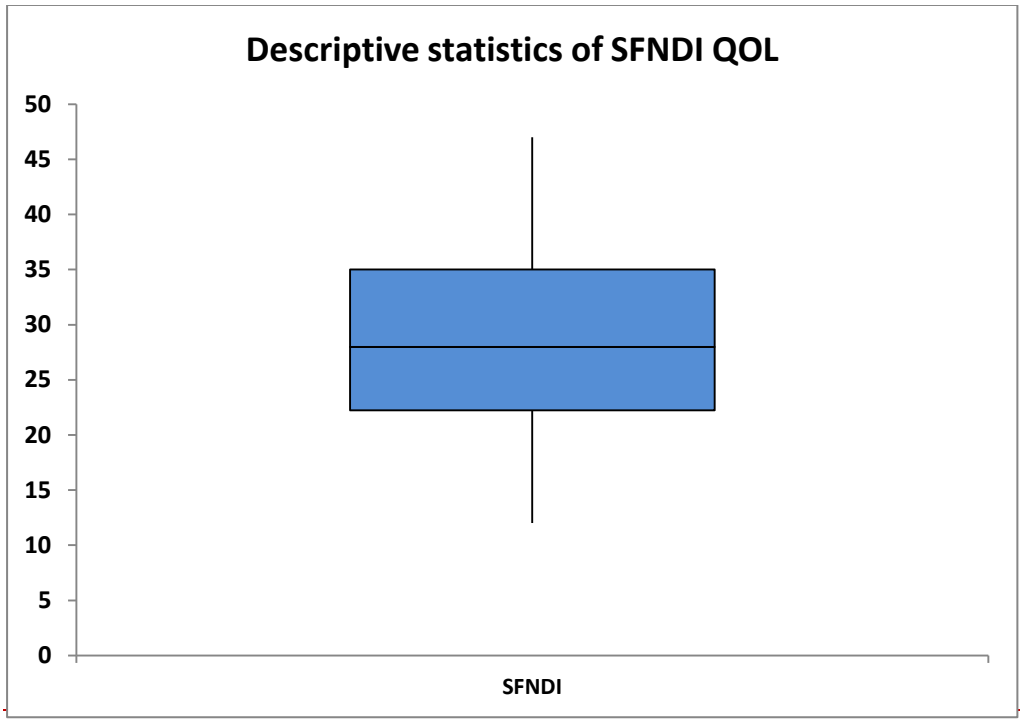

Figure 5.3:-Descriptive statistics of SFNDI QOL QOL.

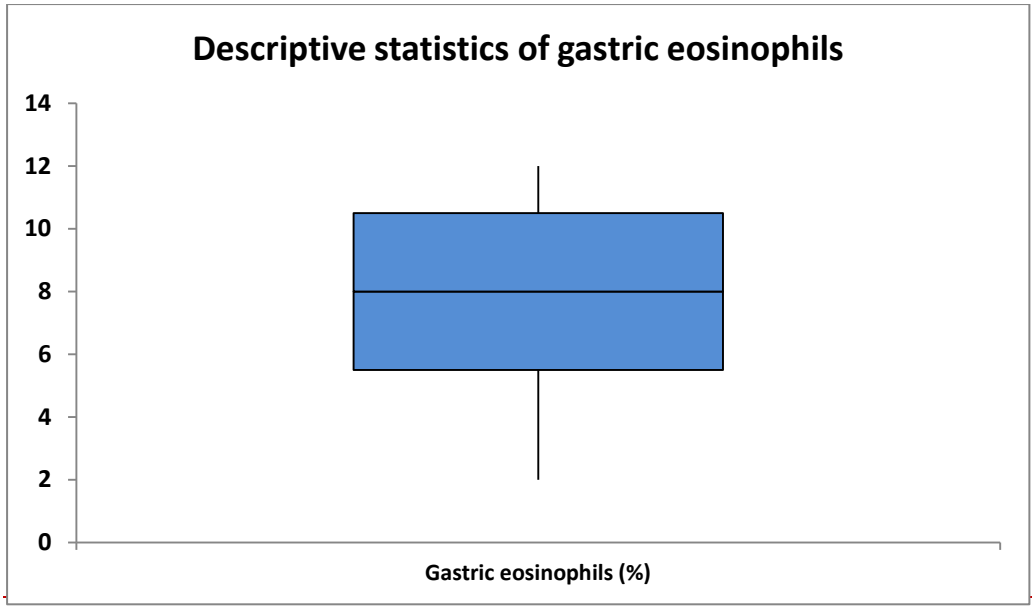

Figure 5.4:-Descriptive statistics of gastric eosinophils (%).

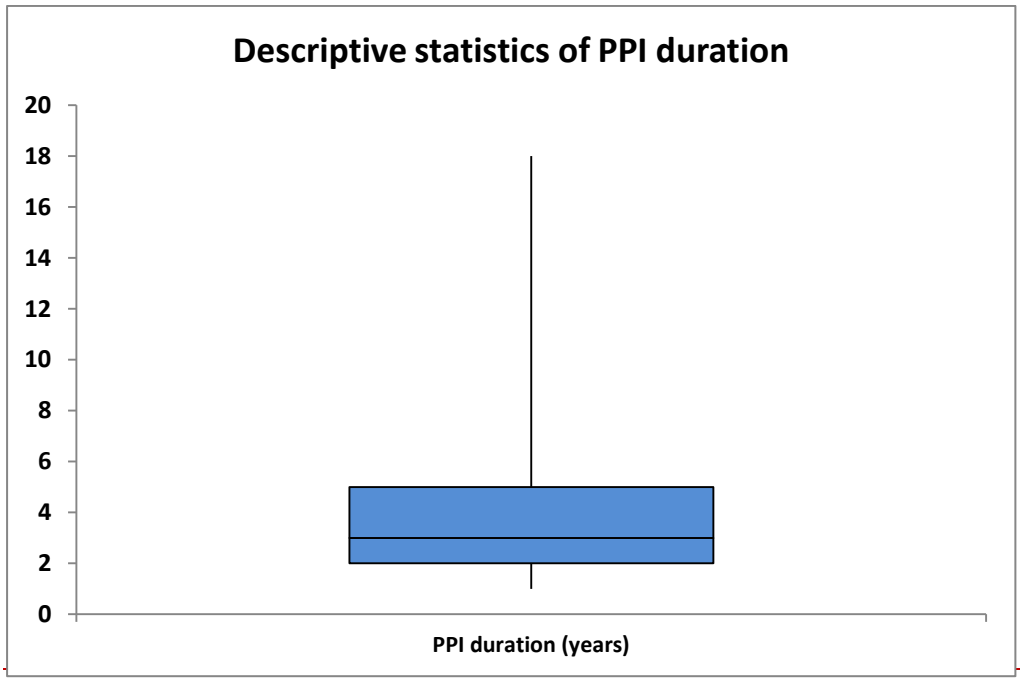

Figure 5.5:-Descriptive statistics of PPI duration.

Mean value of dyspepsia duration among the study subjects was 4.27 ± 3.4 years, with median (25th-75th percentile) of 3 (2-5) years. Mean value of SFNDI QOL QOL among the study subjects was 28.73 ± 8.36, with median (25th-75th percentile) of 28 (22.25-35). Mean value of gastric eosinophils among the study subjects was 7.75 ± 3.62%, with median (25th-75th percentile) of 8 (5.5-10.5)%. Mean value of PPI duration among the study subjects was 3.61 ± 2.6 years, with median (25th-75th percentile) of 3 (2-5) years (Table 5, Figure 5.2 to 5.5).

Table 6:- Descriptive statistics of subgroup score in cases.

Variable	EPS(n52)	PDS (n45)	Overlap (n53)	p-value
Age	37.82 ± 15.86	44.66 ± 20.76	42.50 ± 16.59	A vs B = 0.0719 B vs C = 0.579 A vs C = 0.141
Gender	Males 27 Females 25	Males 27 Females 18	Males 16 Females 37	A vs B = 0.206 B vs C = 0.009 A vs C = 0.009
BMI	24.1 ± 3.1 Kg/m^2	25.3 ± 3.8 Kg/m^2	23.5 ± 2.5 Kg/m^2	A vs B = 0.090 B vs C = 0.006 A vs C = 0.277
Duodenal Eosinophil(/hpf)	19.26 ± 12.15	17.53± 11.17	17.28 ± 10.06	A vs B = 0.469 B vs C = 0.907 A vs C = 0.364
Percentage with raised duodenal eosinophil counts	N=35 (67%)	N=25(55.56%)	N=36(67%)	A vs B = 0.126 B vs C = 0.126 A vs C = 0
Symptom Severity Score	7.42 ± 0.95	7.73 ± 0.809	12.69 ± 1.51	A vs B = 0.089 B vs C <0.0001 A vs C <0.0001
SFNDI QOL QOL	27.55 ± 8.42	29.88 ± 8.08	28.88 ± 8.53	A vs B = 0.169 B vs C = 0.554 A vs C =0.423
Dyspepsia Duration (years)	4.51 ± 4.02	4.11 ± 3.55	4.15 ± 2.57	A vs B = 0.607 B vs C = 0.948 A vs C = 0.585
PPI Duration(years)	3.82 ± 2.63	3.57 ± 3.07	3.43 + 2.10	A vs B = 0.666 B vs C = 0.790 A vs C = 0.402

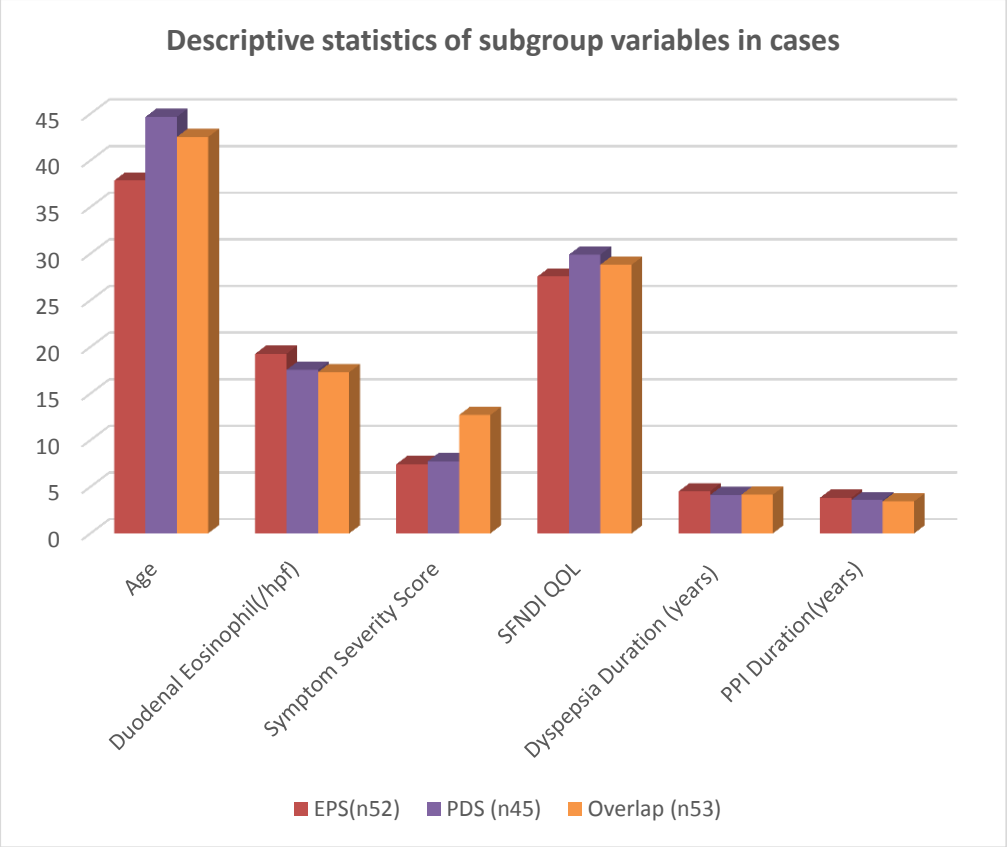

Figure 6 :Descriptive statistics of subgroup variables in cases

Figure representing baseline charecteristics of EPS, PDS and Overlap groups (Table6, figure 6)

Table 7:-Correlation of Duodenal eosinophil count with various parameters in cases.

Variables	Duodenal eosinophil count (/hpf))	
	Correlation coefficient	P value
Age	-0.005	0.947
Dyspepsia (years)	-0.048	0.558
EPS SS	-0.0061	0.966
PDS SS	0.376	<0.0001
Overlap SS	0.282	0.04
Bloating SS	-0.066	0.424
Retrosternal discomfort SS	0.288	0.0004
SFNDI QOL QOL	0.21	0.010
PPI duration (years)	-0.046	0.574
Gastric eosinophils (/hpf))	0.098	0.840

Spearman rank correlation coefficient

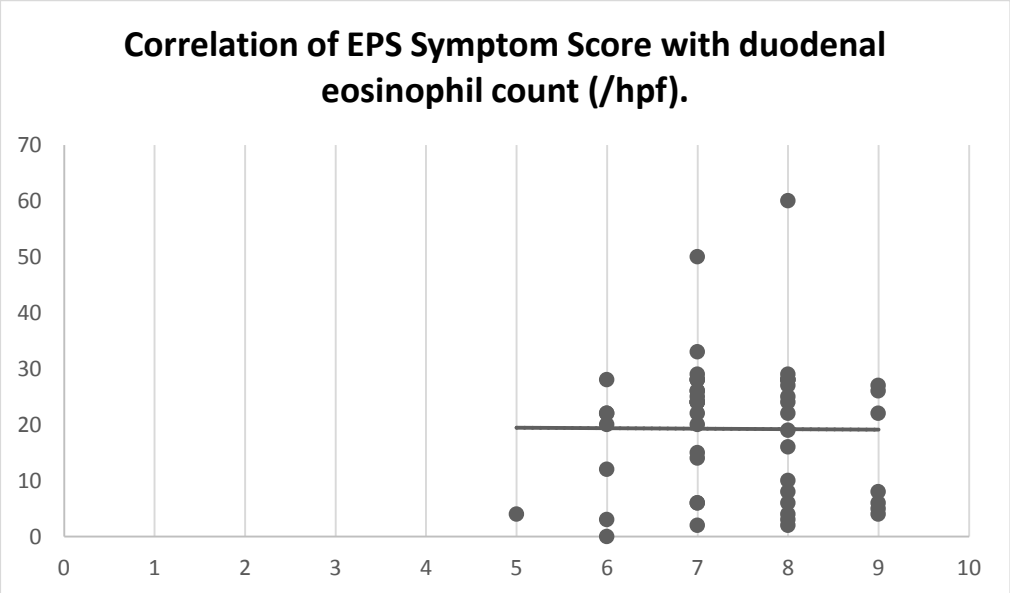

Figure 7.1:-Correlation of EPS Symptom Score with duodenal eosinophil count (/hpf).

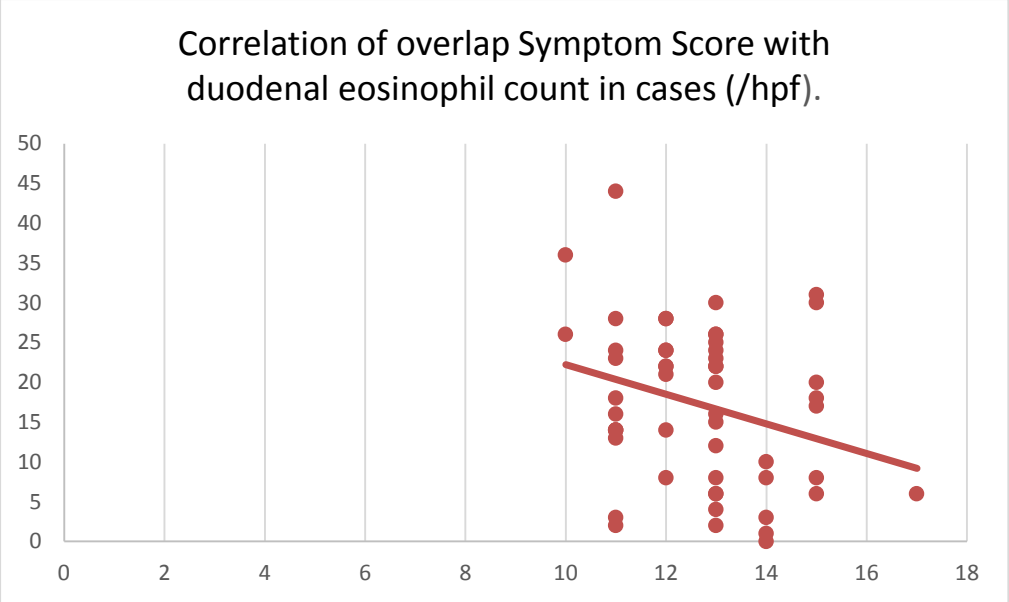

Figure 7.2:-Correlation of overlap Symptom Score with duodenal eosinophil count (/hpf).

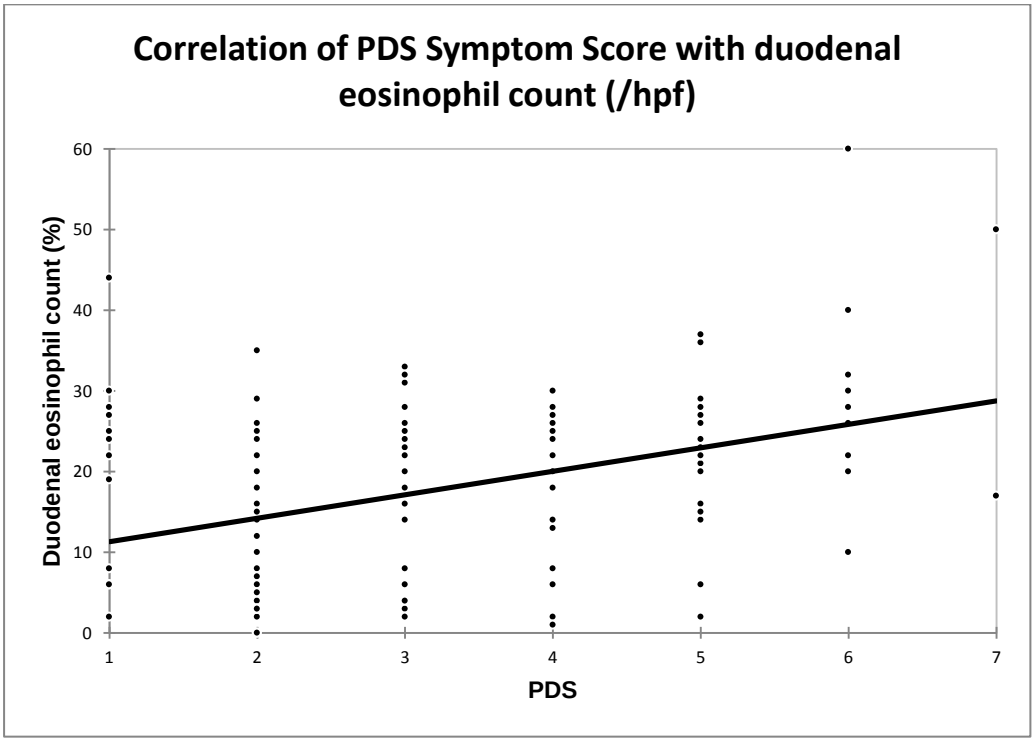

Figure 7.3:-Correlation of PDS Symptom Score with duodenal eosinophil count (/hpf).

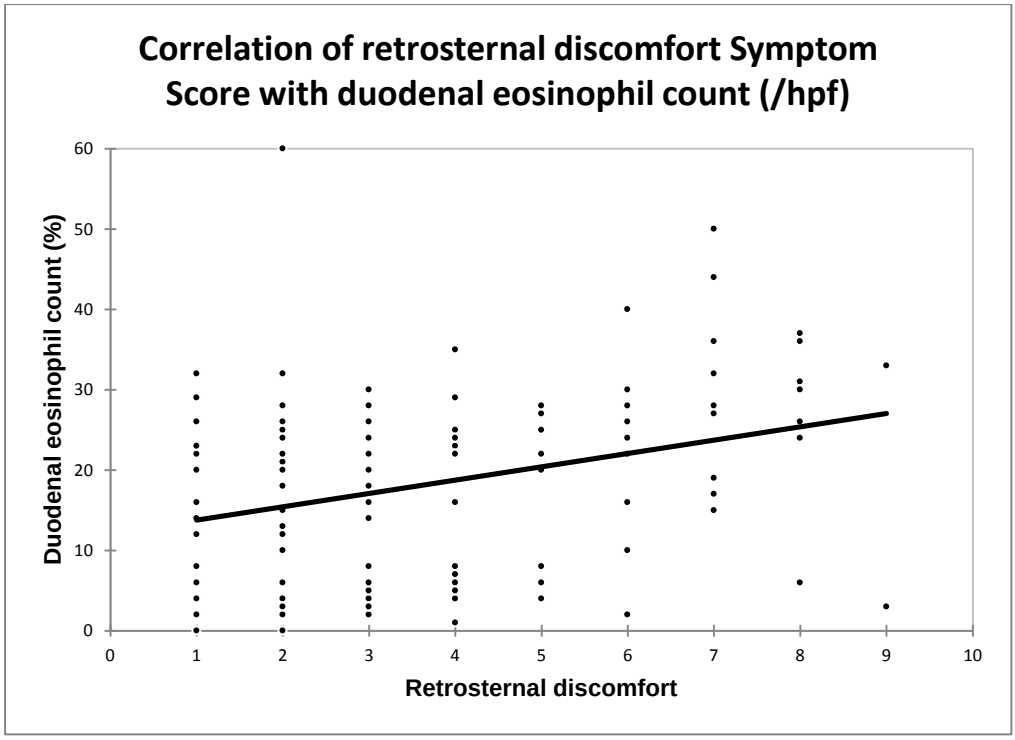

Figure 7.4:-Correlation of retrosternal discomfort Symptom Score with duodenal eosinophil count (/hpf).

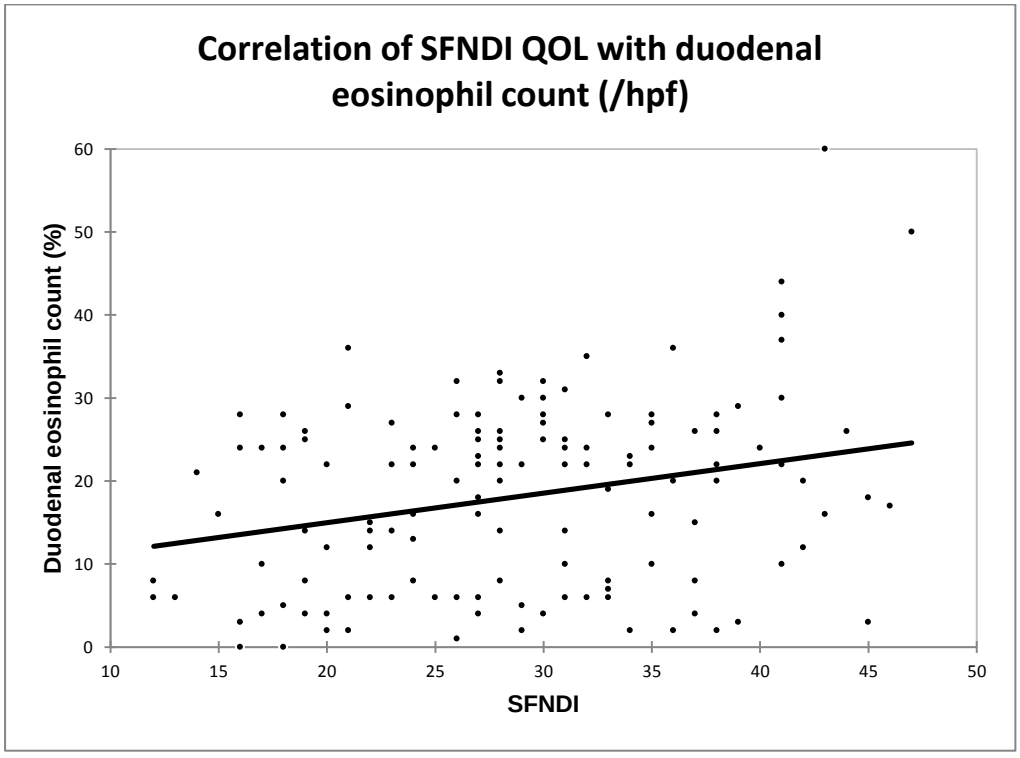

Figure 7.5:-Correlation of SFNDI QOL QOL with duodenal eosinophil count (/hpf).

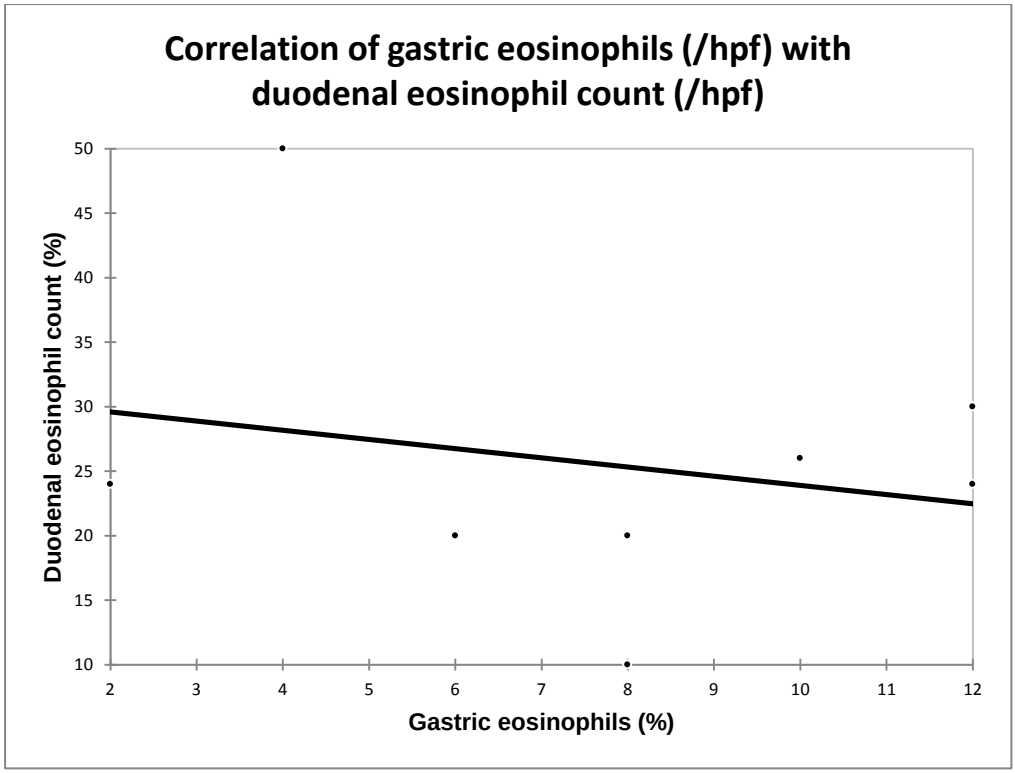

Figure 7.6:-Correlation of gastric eosinophils (/hpf) with duodenal eosinophil count (/hpf) in cases.

Significant weak positive correlation was seen between duodenal eosinophil count (hpf) with PDS, overlap, retrosternal discomfort, SFNDI QOL with correlation coefficient of 0.376, 0.288, 0.21 respectively. Non-significant very weak positive correlation was seen between duodenal eosinophil count (/hpf) with gastric eosinophils (/hpf) with correlation coefficient of 0.098. No correlation was seen between duodenal eosinophil count (/hpf) with dyspepsia (years), PPI duration (years) with correlation coefficient of -0.048, -0.046 respectively. Non-significant very weak negative correlation was seen between duodenal eosinophil count (/hpf) with EPS, bloating with correlation coefficient of -0.0061, -0.282, -0.108, -0.066 respectively (Table 7, figure 7.1 to 7.6).

Table 8:-Association of dyspepsia subgroups symptom score with duodenal eosinophil in cases.

Dyspepsia subgroups	**Normal Duodenal eosinophil** Mean ± SD	**Raised duodenal eosinophil** Mean ± SD	**P value**
EPS (n 53)	7.52 ± 1.23	7.38 ± 0.81	0.628‡
PDS(n 45)	6.5 ± 1.45	7.36 ± 0.99	0.002‡
Overlap(n 52)	13.47 ± 1.46	12.33 ± 1.41	0.009‡
Bloating(n 61)	4.68 ± 1.46	4.72 ± 1.67	0.437‡
Retrosternal discomfort(n 61)	5.04 ± 1.75	5.91 ± 2.19	0.006‡

‡ Independent t test

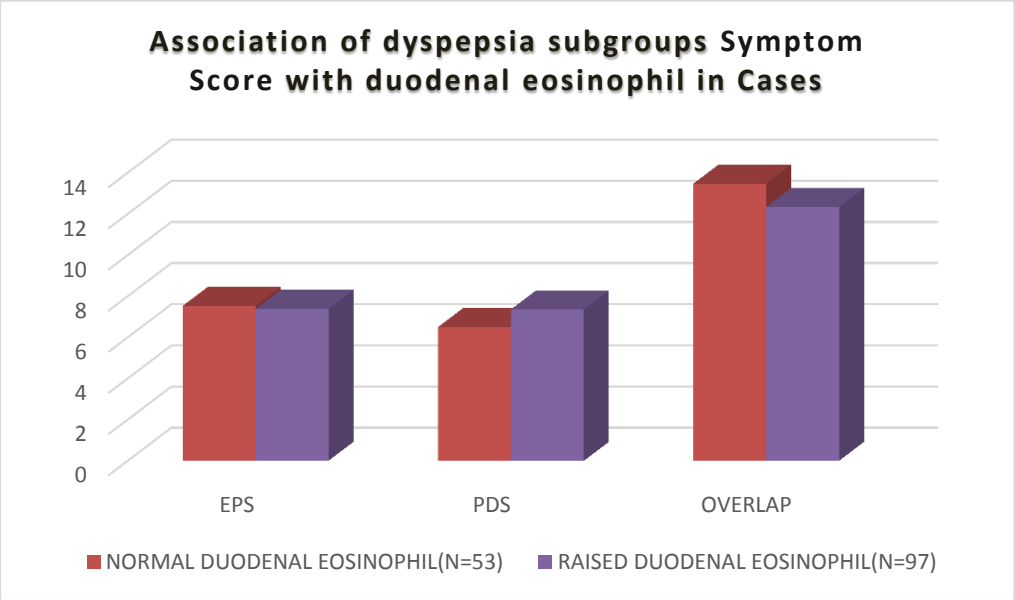

Figure 8:-Association of dyspepsia subgroups Symptom Score with duodenal eosinophil in cases.

No significant association was found in dyspepsia subgroups with duodenal eosinophil: EPS symptom score (7.52 ± 1.23 in raised vs. 7.38 ± 0.81 in normal, p value = 0.628). Significant association was seen in subgroups PDS (6.5 ± 1.45 vs. 7.36 ± 0.99, p value = 0.002), overlap (13.47 ± 1.46 vs. 12.33 ± 1.41, p value = 0.009) with significantly higher values in raised group (Table 8, Figure 8).

Table 9:-Association of various parameters with duodenal eosinophil in cases

Variable	Normal duodenal eosinophil(n=53) Mean ± SD	Raised duodenal eosinophil(n=97) Mean ± SD	Total	P value
Dyspepsia duration(years)	4.15 ± 2.56	4.33 ± 3.81	4.27 ± 3.41	0.613 §
SFNDI QOL QOL	26.72 ± 8.54	29.82 ± 8.1	28.73 ± 8.36	0.029 ‡
Gastric eosinophils	8 ± 0	7.71 ± 3.9	7.75 ± 3.62	0.948 ‡
PPI use duration	3.74 ± 2.19	3.55 ± 2.81	3.61 ± 2.6	0.237 §

§ **Mann Whitney test** ‡ **Independent t test**

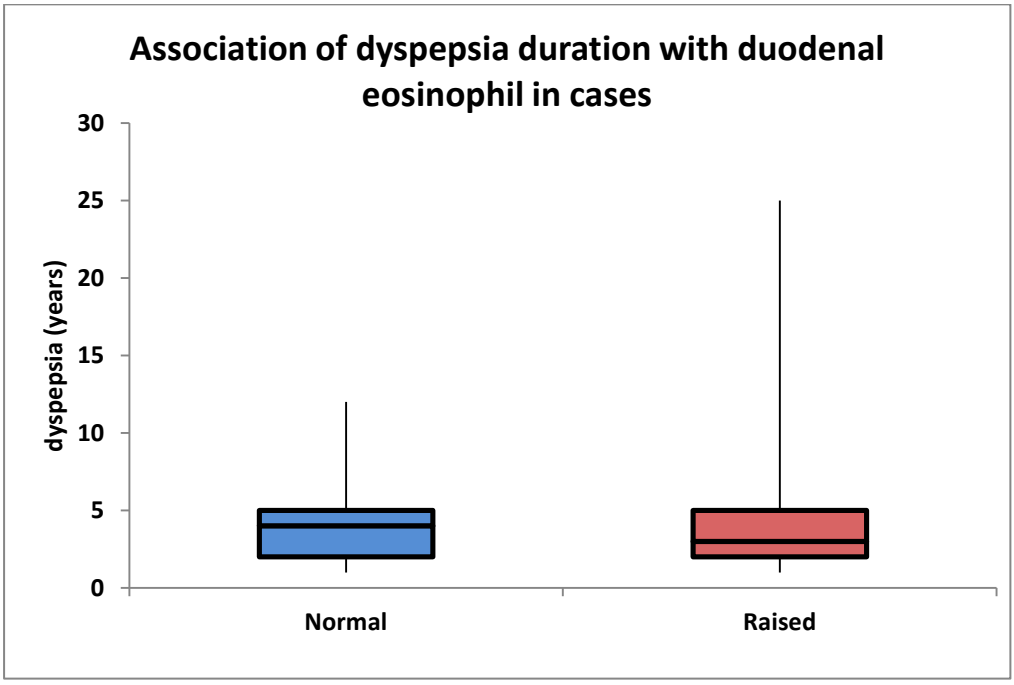

Figure 9.1:-Association of dyspepsia duration with duodenal eosinophil in cases (non-parametric variable, Box-whisker plot)

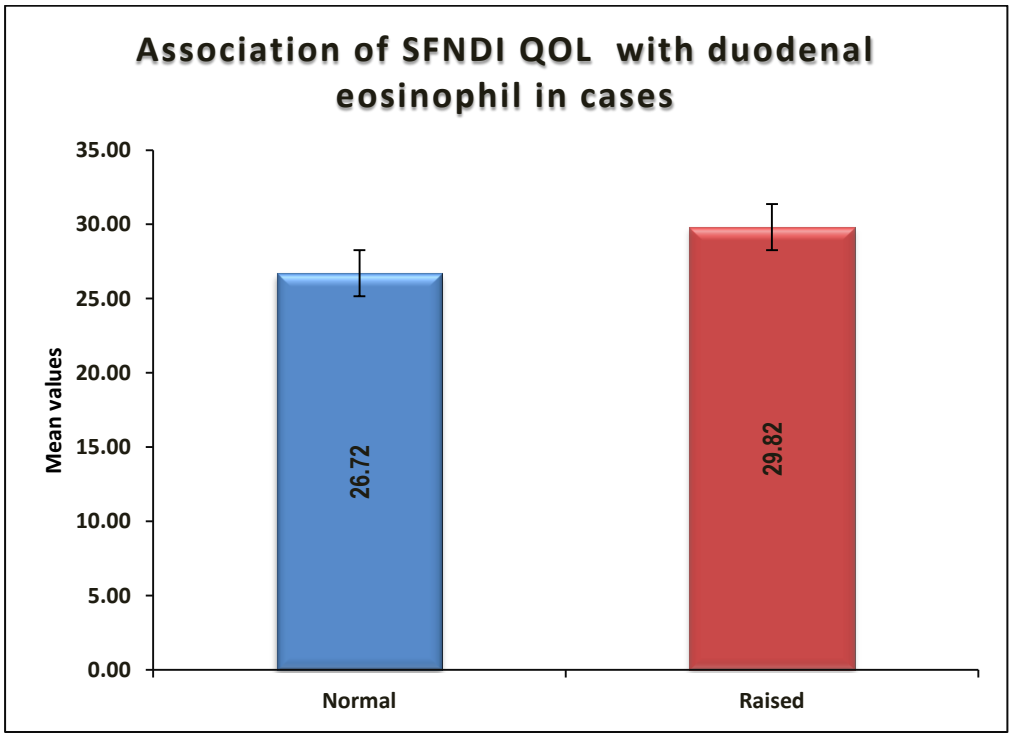

Figure 9.2:-Association of SFNDI QOL QOL with duodenal eosinophil in cases.

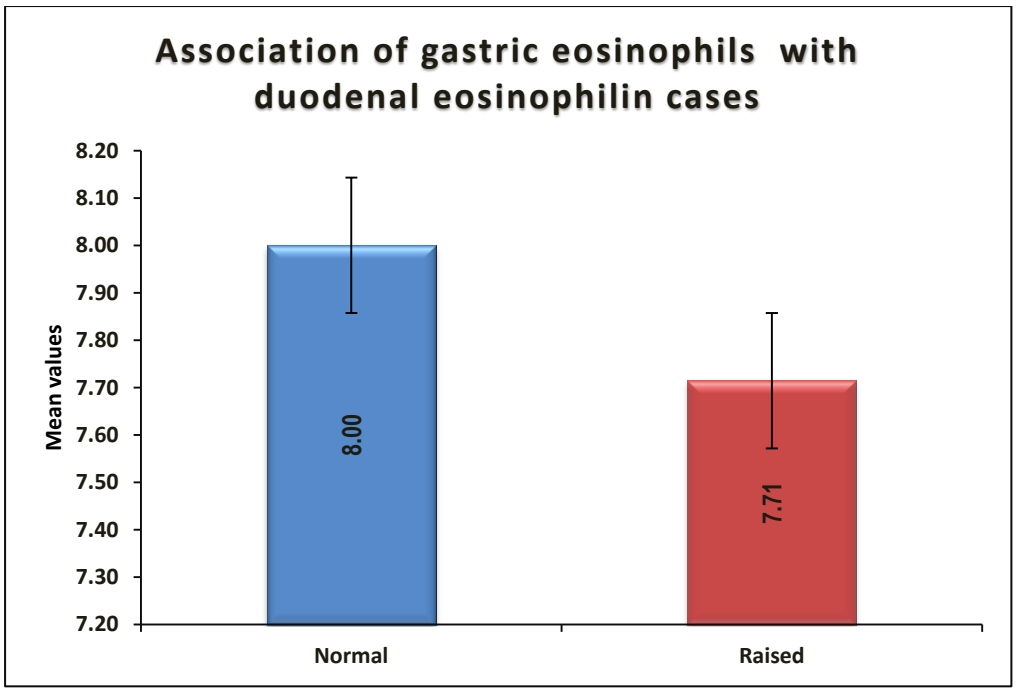

Figure 9.3:-Association of gastric eosinophils with duodenal eosinophil in cases.

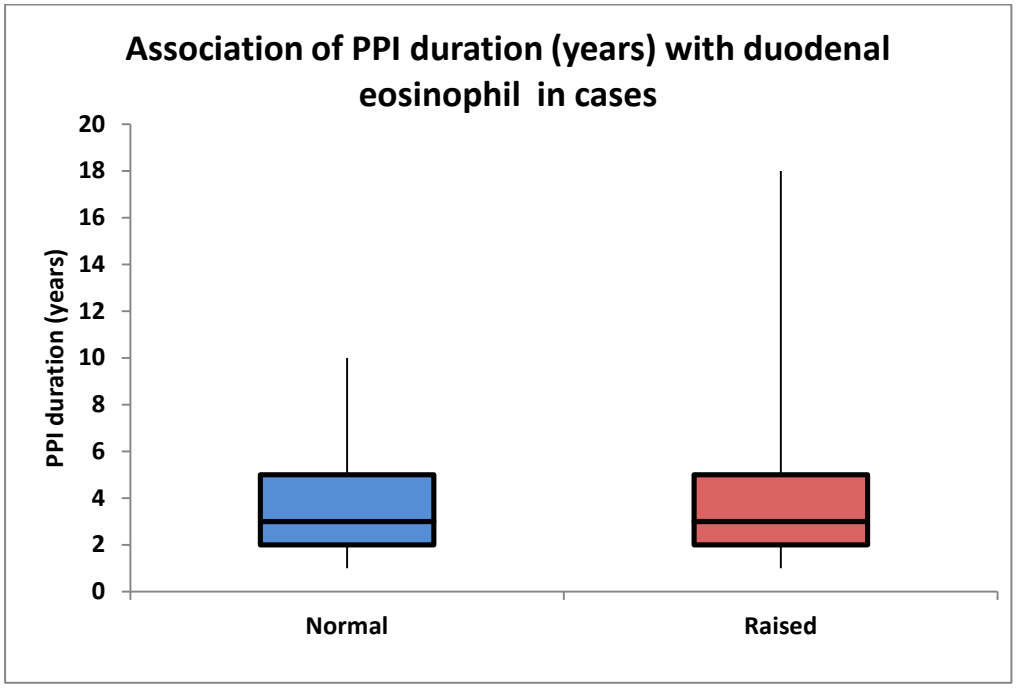

Figure 9.4:-Association of PPI duration with duodenal eosinophil in cases.(non-parametric variable, Box-whisker plot)

Mean of dyspepsia duration was 4.15 ± 2.56 years in raised duodenal eosinophil group and 4.33 ± 3.81years in normal duodenal eosinophil group, with no significant association between them (p value = 0.613). Mean ± SD of gastric eosinophils was 7.71 ± 3.9% in raised duodenal eosinophil group and 8 ± 0% in normal duodenal eosinophil group, with no significant association between them (p value = 0.948). Compared to cases with normal duodenal eosinophil group, cases with raised duodenal eosinophil group had significantly higher mean ± SD of SFNDI QOL (29.82 ± 8.1 vs. 26.72 ± 8.54, p value = 0.029). Mean PPI duration was 3.74 ± 2.19 years in normal and 3.55 ± 2.81 in raised duodenal eosinophil groups, with no significant association between them (p value = 0.237) (Table 9, Figure 9.1 to 9.4).

Table 10:-Comparison of degranulation between cases and controls.

Degranulation	Cases(n=150)	Controls(n=50)	Total	P value
No	115 (76.67%)	50 (100%)	165 (82.50%)	<.0001*
Yes	35 (23.33%)	0 (0%)	35 (17.50%)	
Total	150 (100%)	50 (100%)	200 (100%)	

* **Fisher's exact test**

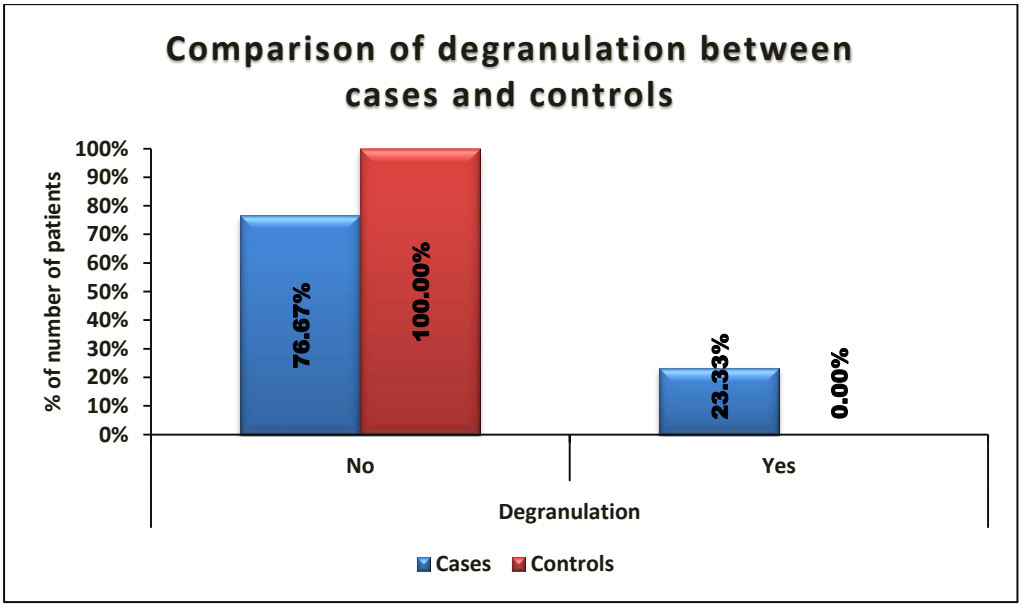

Figure 10:-Comparison of degranulation between cases and controls.

Compared to controls, cases had significantly higher proportion of patients with degranulation: 23.33% of cases vs. 0% of controls. Conversely, proportion of patients without degranulation was significantly lower in cases compared to controls (76.67% vs. 100%) (p value < 0.0001) (Table 10, Figure 10).

Table 11:-Association of duodenal eosinophil with degranulation in Cases.

Duodenal eosinophil (per hpf)	Cases With degranulation(n=35)	Cases Without degranulation(n=115)	Total	P value
Mean ± SD	23.17 ± 12.35	16.53 ± 10.24	18.08 ± 11.09	0.012 §

§ **Mann Whitney test**

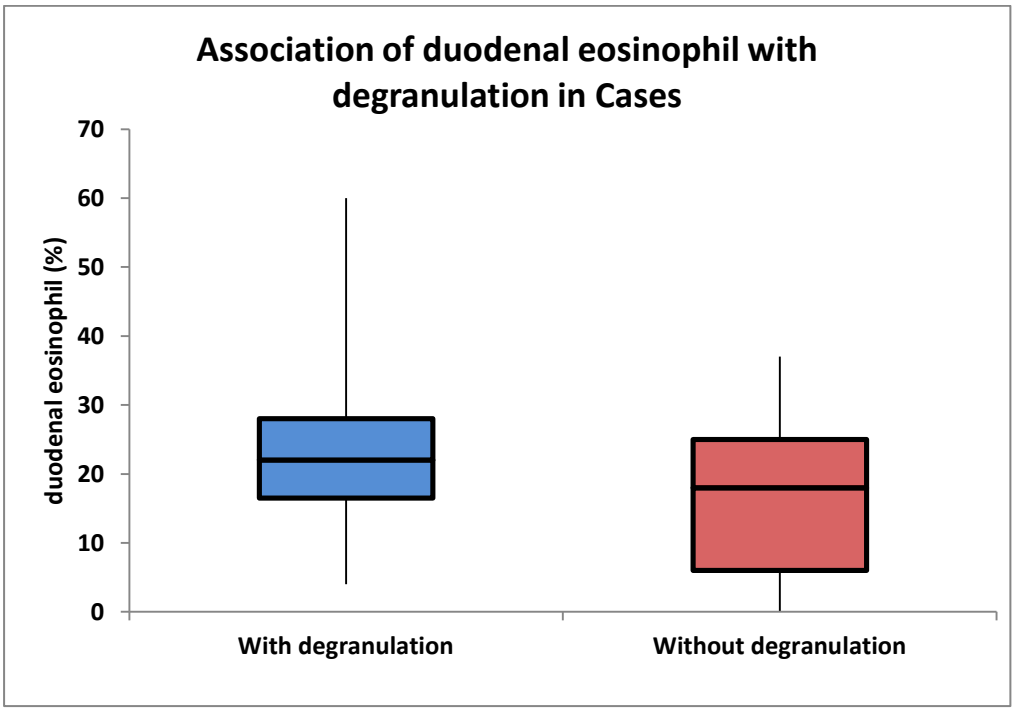

Figure 11:-Association of duodenal eosinophil with degranulation in Cases.(non-parametric variable, Box-whisker plot)

Mean of duodenal eosinophil in cases with degranulation was 23.17 ± 12.35/hpf which was significantly higher compared to cases without degranulation 16.53 ± 10.24/hpf) (p value = 0.012) (Table 11, Figure 11).

Table 12:-Association of dyspepsia subgroups Symptom Score with degranulation in cases.

Dyspepsia subgroups Symptom Score	With degranulation(n=35)	Without degranulation(n=115)	P value
EPS SS	7.25 ± 0.96	7.47 ± 0.96	0.397‡
PDS SS	7.53 ± 0.77	6.71 ± 1.32	0.042 ‡
Overlap SS	12.69 ± 1.52	12.7 ± 1.6	0.984‡
Bloating SS	4.97 ± 1.77	4.71 ± 1.64	0.421‡
Retrosternal discomfort SS	5.17 ± 2.39	4.93 ± 1.96	0.920

‡ Independent t test

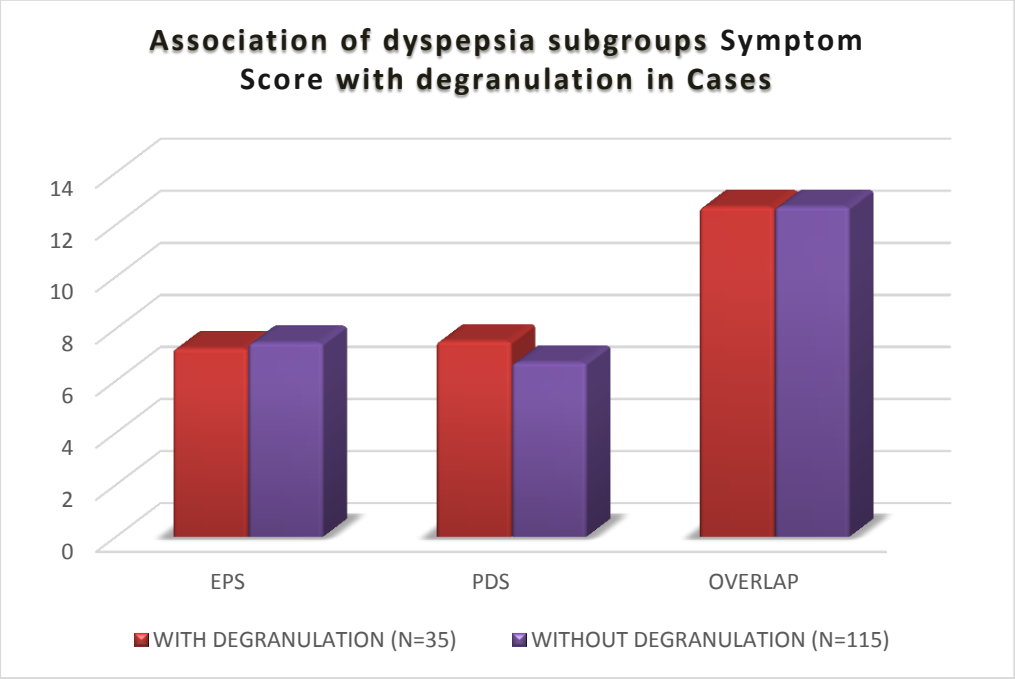

Figure 12:-Association of dyspepsia subgroups Symptom Score with degranulation in cases.

No significant association was found in dyspepsia subgroups symptom score with degranulation: EPS (7.25 ± 0.96 in those with degranulation vs. 7.47 ± 0.96 in those without degranulation, p value = 0.397), overlap (12.69 ± 1.52 vs. 12.7 ± 1.6, p value = 0.984). Significant

association was observed in PDS (7.53 ± 0.77 vs. 6.71 ± 1.32, p value = 0.042). (Table 12, Figure 12).

Table 13:-Association of duration of dyspepsia and SFNDI QOL QOL in cases with or without degranulation.

VARIABLE	With degranulation(n=35) Mean ± SD	Without degranulation(n=115) Mean ± SD	Total	P value
Dyspepsia Duration (years)	3.8 ± 2.63	4.41 ± 3.61	4.27 ± 3.41	0.471 §
SFNDI QOL QOL	32.66 ± 9.08	27.53 ± 7.78	28.73 ± 8.36	0.001 ‡

§ Mann Whitney test ‡ Independent t test

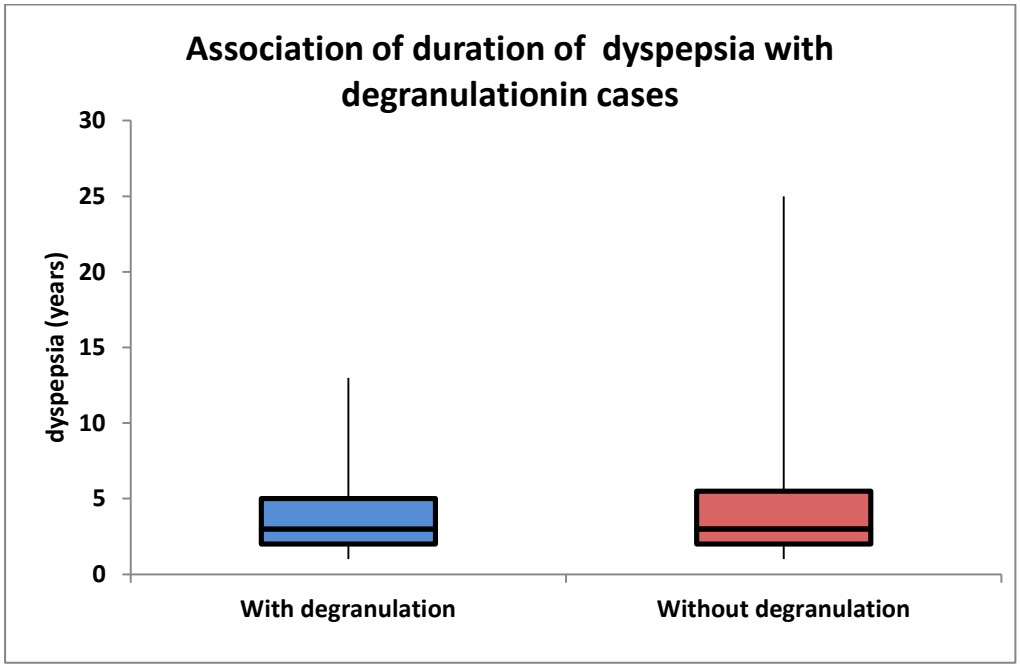

Figure 13.1:-Association of duration of dyspepsia with degranulation in cases.(non-parametric variable, Box-whisker plot)

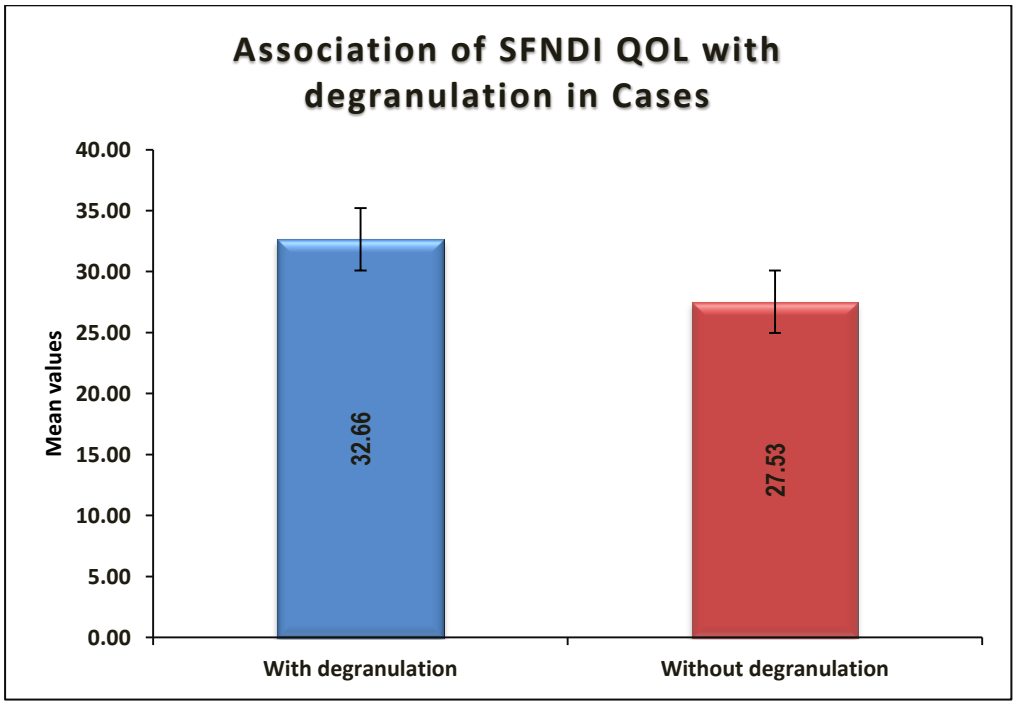

Figure 13.2:-Association of SFNDI QOL QOL with degranulation in Cases.

Mean of dyspepsia duration was 3.8 ± 2.63 years in those with degranulation and 4.41 ± 3.61 years in those without degranulation, with no significant association between them (p value = 0.471) Mean ± SD of SFNDI QOL QOL in patients with degranulation was 32.66 ± 9.08, which was significantly higher compared to those without degranulation (27.53 ± 7.78) (p value = 0.001) (Table 13, Figure 13.1 and 13.2).

Table 14:-Association of degranulation with duodenal eosinophil in cases.

Degranulation	Normal duodenal eosinophil(n=53)	Raised duodenal eosinophil(n=97)	Total	P value
No	46 (86.79%)	69 (71.13%)	115 (76.67%)	0.03†
Yes	7 (13.21%)	28 (28.87%)	35 (23.33%)	
Total	53 (100%)	97 (100%)	150 (100%)	

† **Chi square test**

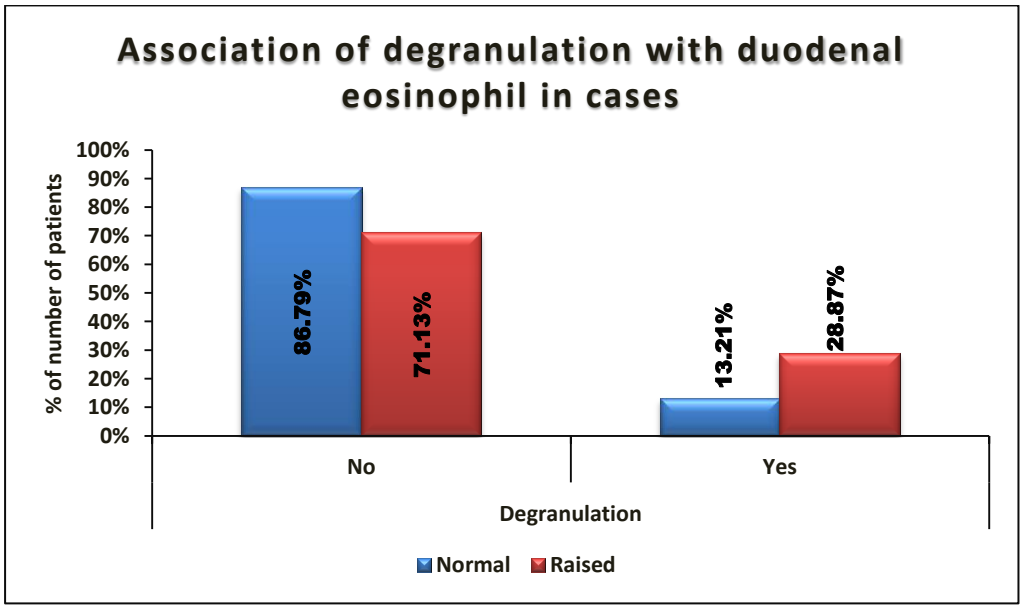

Figure 14:-Association of degranulation with duodenal eosinophil in cases.

Compared to normal eosinophil group in cases, cases in raised group had significantly lower proportion of patients without degranulation (71.13% vs. 86.79%) and significantly higher proportion of patients with

degranulation (28.87% vs. 13.21%) (p value = 0.03) (Table 14, Figure 14).

Table 15:-Comparison of co-morbidities, personal history and medications between cases and controls.

Co-morbidities	Cases(n=150)	Controls(n=50)	Total	P value
Hypertension	24 (16%)	9 (18%)	33 (16.50%)	0.741†
Diabetes mellitus	18 (12%)	5 (10%)	23 (11.50%)	0.701†
Asthma	5 (3.33%)	0 (0%)	5 (2.50%)	0.334*
SSRI	6 (4%)	1 (2%)	7 (7%)	0.252
TCA	5 (3.33%)	2 (4%)	7 (3%)	0.407†

*** Fisher's exact test, † Chi square test**

Table 16:-Association of duodenal eosinophil with comorbidities, and medications in Cases.

VARIABLE		Median Eosinophil count	P-value
HYPERTENSION	YES (n=24)	17.5 (5.75-24)	0.283§
	NO (n=126)	20 (8-26)	
DIABETES	YES (n=18)	20 (6.5-25.75)	0.935§
	NO (n=132)	20 (7.25-26)	
ASTHMA	YES (n=5)	22 (20-25)	0.615§
	NO (n=145)	20 (7.25-26)	
	NO (n=144)	20 (6.75-26)	
SSRI USE	YES (n=6)	22 (22-23.5)	0.437§
	NO (n=144)	20 (6.75-26)	
TCA USE	YES (n=5)	20 (17-21)	0.671§
	NO (n=145)	20 (7-26)	

§ **Mann Whitney test**

Table 17:-Association of co-morbidities and medications with raised and normal duodenal eosinophil in cases

Co-morbidities	Normal duodenal eosinophil(n=53)	Raised duodenal eosinophil(n=97)	Total	P value
Hypertension	10 (18.87%)	14 (14.43%)	24 (16%)	0.479†
Diabetes mellitus	7 (13.21%)	11 (11.34%)	18 (12%)	0.737†
Asthma	1 (1.89%)	4 (4.12%)	5 (3.33%)	0.657*
SSRI	1 (1.89%)	5 (5.15%)	6 (4%)	0.42
TCA	1 (1.89%)	4 (4.12%)	5 (3.33%)	0.657

*** Fisher's exact test, † Chi square test**

Table 18:-Correlation of gastric eosinophils with various parameters in cases.

Variables	Gastric eosinophils (/hpf)	
	Correlation coefficient	P value
Dyspepsia (years)	-0.453	0.243
EPS SS	0.257	0.582
PDS SS	-0.127	0.752
Overlap SS	0.279	0.536
Bloating SS	0.393	0.360
Retrosternal discomfort SS	0.497	0.243
SFNDI QOL QOL	-0.53	0.171

Spearman rank correlation coefficient

Table 19:-Univariate logistic regression to find out significant risk factors of raised duodenal eosinophil in cases.

Variable	Beta coefficient	Standard error	P value	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
EPS SS	0.010	0.015	0.506	1.065	-0.020	0.414
PDS SS	0.623	0.141	<0.0001	1.865	1.413	2.460
Overlap SS	-0.063	0.020	0.003	0.972	-0.104	-0.021
Bloating SS	0.049	0.104	0.637	1.050	0.856	1.288
Retrosternal discomfort SS	0.213	0.092	0.020	1.238	1.034	1.481
SFNDI QOL QOL	0.045	0.021	0.035	1.046	1.003	1.091

On performing univariate regression, overlap symptom score, PDS SS, retrosternal discomfort SS , SFNDI QOL QOL score were significantly higher in patients with raised duodenal eosinophil. With the increase in PDS SS, overlap symptom score, retrosternal discomfort symptom scores and SFNDI QOL QOL score, risk of raised duodenal eosinophil significantly increases with odds ratio of 1.865(1.413 to 2.46), 1.238(1.034 to 1.481), 1.046(1.003 to 1.091) respectively (Table 19).

Table 20:- Multivariate logistic regression to find out significant risk factors of raised duodenal eosinophil in cases.

Variable	Beta coefficient	Standard error	P value	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
PDS SS	0.655	0.150	<0.0001	1.925	1.435	2.580
Retrosternal discomfort SS	0.162	0.102	0.112	1.176	0.963	1.435
SFNDI QOL QOL	0.047	0.025	0.062	1.048	0.998	1.101

On performing multivariate regression, PDS SS was independently associated with raised duodenal eosinophil after adjusting for confounding factors. With the increase in PDS SS, risk of raised duodenal eosinophil significantly increases with adjusted odds ratio of 1.925(1.435 to 2.58) (Table 20).

DISCUSSION

The study included a total of 200 participants, consisting of 150 individuals diagnosed with functional dyspepsia (FD) and 50 adults serving as controls. No statistically significant differences were found in the age distribution study between cases and controls across distinct age categories (≤20 years, 21-40 years, 41-60 years, >60 years). The average age of the FD group was 40.88 years, which was somewhat lower than the average age of 42.79 years achieved by the control group. However, this difference did not reach statistical significance ($p = 0.531$). The findings regarding duodenal eosinophil levels were not confounded by age.

The study group consisted of 53.33% female cases with FD, compared to 42% of controls. While the proportion of females in the FD group was greater, this disparity did not reach statistical significance ($p = 0.165$), suggesting that gender is improbable to have affected the main results of the study.

The study investigated the presence of comorbidities like hypertension, diabetes mellitus, and asthma and identified no statistically significant differences between those with FD and the control group. The prevalence of hypertension was 16% in subjects with FD and 18% in controls ($p = 0.741$). Diabetes mellitus was found in 12% of FD cases and 10% of controls ($p = 0.701$), while asthma was detected in 3.33% of FD cases and 0% of controls ($p = 0.334$). The results indicate that these concurrent disorders were equally prevalent in both groups and probably did not complicate the connection between FD and duodenal eosinophilia.

The duration of functional dyspepsia (FD) varied, with an average duration of 4.27 ± 3.4 years and a middle duration of 3 years. The enduring experience of dyspeptic symptoms underscores the persistent

character of FD in the group under investigation. Utilization of proton pump inhibitors (PPIs), a frequently used therapy for dyspepsia, was documented to have an average duration of 3.61 ± 2.6 years. These findings indicate that the majority of patients with FD in our study group were receiving prolonged treatment with PPIs, highlighting the persistent nature of FD. Furthermore, this finding emphasizes the deficiencies of the existing therapy choices and the necessity to further investigate the comprehension of the varied unidentified pathophysiological processes that contribute to the disease in order to create precise and efficient remedies. A comparatively elevated SFNDI QOL score in our patients provides additional evidence of the influence of different symptoms on quality of life and decreased productivity or output in the workplace. This, in addition to the ongoing symptoms despite sufficient regular PPI treatment, emphasizes the need to conduct studies in these patients.

The use of tricyclic antidepressants (TCA) was observed in 3.33% of cases with FD and 4% of controls ($p = 0.061$), whereas the use of selective serotonin reuptake inhibitors (SSRI) was recorded in 4% of cases with FD and 2% of controls ($p = 0.107$). These results indicate that any variations in the usage of psychotropic drugs do not seem to have a substantial impact on the main results of the study.

A standardized threshold for duodenal eosinophils in the healthy population has not yet been established, despite a considerable duration since the initial evidence of their association with FD. Although numerous studies, including reviews and meta-analyses, have been undertaken on this topic, they are constrained by a small sample size and unidentified ethnic and geographical differences in duodenal eosinophil measurement. One further limitation of the several efforts to establish the normal range of duodenal eosinophils is the reliance on mean or median with standard deviation, which is now seen as an excessively simplistic approach for such derivation. Furthermore, as we were unable to find a comparable study from India despite thorough

research, we instead obtained it from our control group. An important finding of our study is the determination of a threshold value for duodenal eosinophil levels in the healthy population. Our study established a threshold of 12/hpfeosinophils, to distinguish between healthy and abnormal values. The area under the receiver operating characteristic (ROC) curve for this cut-off was 0.749, suggesting strong discriminatory power ($p < 0.0001$), along with high sensitivity, specificity, and accuracy in differentiating between healthy and affected persons. This threshold could function as a valuable diagnostic instrument for identifying patients with FD who may derive advantages from precision therapies.

Our work makes a noteworthy contribution to the field by establishing a duodenal eosinophil cut-off (>12/hpf) for the healthy population. Underpinned by the ROC analysis, this threshold offers a pragmatic diagnostic instrument that may be applied in clinical environments. Prior research proposed different thresholds for eosinophilia. [49,54,65.57.65,70,86,87]. The greater duodenal eosinophil count seen in patients with FD compared to controls aligns with previous research conducted in Western populations. Eosinophils have been proposed to be the focus of future therapeutic intervention. [48.53,89] Our data supports the fact that duodenal eosinophilia may be an important therapeutic target in a subset of dyspepsia subjects. However, regional variations in nutrition, lifestyle, and genetics may impact the precise threshold levels.

Taking the cut-off of more than 12 eosinophils per hpf in duodenum, duodenal eosinophilia was seen in 97 out of 150 cases, which is 64.67% of the enrolled functional dyspepsia patients.

An important observation of this study is the markedly elevated duodenal eosinophil count in individuals with FD in comparison to the control group. The mean duodenal eosinophil count in patients with FD was 18.08 ± 11.09/hpf, whereas in controls it was 8.6 ± 7.66/hpf. This

difference was statistically significant ($p < 0.0001$). This finding provides evidence in favor of the premise that duodenal eosinophilia is a prominent characteristic of FD and could contribute to its development.

We also investigated the correlation between duodenal eosinophil levels and other baseline parameters. Our study revealed statistically significant correlation between duodenal eosinophil count and PDS symptom score, overlap symptom score, retrosternal discomfort symptom scores and SFNDI QOL (quality of life) scores (correlation coefficient of 0.376, 0.282, 0.288 and 0.21 with p-value of <0.0001, 0.04, 0.0004 and 0.01 respectively) whereas no statistically significant associations were found between duodenal eosinophil counts and age, duration of dyspepsia, EPS symptom score, bloating or PPI duration (with correlation coefficient = -0.005,-0.048, -0.0061, -0.066, -0.046 with p-values of 0.947, 0.558, 0.966, 0.424, 0.574 respectively). Our finding provides additional support for the involvement of eosinophilic infiltration in the duodenal mucosa in the development and continuation of disease, especially in PDS subgroup and associated with higher incidence of retrosternal discomfort and poorer quality of life but independent of age, duration of symptoms, EPS symptoms and duration of PPI use.

Several intriguing patterns were seen when comparing FD individuals with normal vs elevated duodenal eosinophil levels. A statistically significant difference was seen in PDS and overlap symptom scores between FD patients with elevated eosinophil counts (mean = 6.5 ± 1.45 and 13.47 ± 1.46)/hpf and those with normal counts (mean = 7.36 ± 0.99 and 12.33 ± 1.41)/hpf with $p < 0.002$ and 0.009 respectively. Furthermore, patients with elevated eosinophils had a higher incidence of retrosternal discomfort symptom score (mean = 5.04 ± 1.75)/hpf compared to those with normal counts (mean = 5.91 ± 2.19)/hpf with p value of 0.006. Based on these results, it appears that duodenal

eosinophilia is specifically linked to PDS and these dyspeptic symptoms, which may reflect a more inflammatory phenotype of FD.

When comparing the two groups, no notable disparities were seen in terms of epigastric pain syndrome (EPS), bloating, or overlap symptoms. Consequently, although eosinophilia serves as an indicator of inflammation in FD, it may not be the exclusive factor responsible for all the symptoms associated with FD.

In this study, the napean dyspepsia index short form score (SFNDI QOL) is the primary measure employed to evaluate the severity of dyspeptic symptoms. SFNDI QOL is a rigorously approved and validated questionnaire used to evaluate used to evaluate the severity of symptoms across multiple population in the world, including Indian population. Among the FD group, the average SFNDI QOL score was 28.73 ± 8.36, with a median score of 28. FD patients with elevated duodenal eosinophils had a higher SFNDI QOL score (mean = 29.82 ± 8.2) compared to those with normal counts (mean = 26.72 ± 8.54) with p value of 0.029. A measurable assessment of symptom burden in the FD population especially in patients with raised duodenal eosinophil count is provided by these scores, which are crucial for elucidating the relationship between symptom severity and duodenal eosinophil levels.

On comparing dyspepsia patients with normal and elevated duodenal eosinophil counts, we found that increased eosinophil levels were associated with a more severe clinical manifestation. In particular, patients who had elevated levels of eosinophils showed increased occurrences of PDS and retrosternal pain along with higher SFNDI QOL scores. These findings are consistent with the study conducted by Talley et al. (2007), who initially proposed that duodenal eosinophilia may serve as an indicator for more severe types of FD.[49] In a similar vein, Du et al. (2016) discovered that individuals with eosinophilia frequently suffer more severe gastrointestinal symptoms, therefore supporting the idea that eosinophils play a role in the development of

FD, especially in patients who experience mostly PDS and retrosternal symptoms.[60]

In line with existing data like study conducted by Walker et al. (2014) which documented extensive link between duodenal eosinophilia and different subtypes of functional dyspepsia (FD),[58] our study suggested a statistically significant relationship between increased eosinophil levels and symptoms such as postprandial distress syndrome (PDS) but not epigastric pain syndrome (EPS), though some studies suggest no correlation between duodenal eosinophils and dyspepsia subtypes. This underscores that the observed differences may be attributed to variations in the groups of individuals included in the studies, the criteria used for diagnosis, or the possible influence of other processes, such as visceral hypersensitivity or gastric motility disorders, which may have a more substantial impact on these specific subtypes. [77]

A comprehensive analysis of dyspepsia patients with normal and elevated duodenal eosinophil levels highlighted certain significant distinctions. As indicated before, increased eosinophil levels were linked to more frequent episodes of PDS, overlap syndrome and retrosternal pain. Moreover, these individuals exhibited elevated SFNDI QOL values, suggesting a more pronounced total symptom burden and inferior quality of life. The discovery of these connections emphasizes the possible usefulness of duodenal eosinophil counts as a biomarker for identifying a subgroup of patients with refractory FD who display a more severe or inflammatory phenotype. This presents novel opportunities for investigation of therapies for such patients.

Furthermore, the involvement of gastric eosinophils in FD was investigated. A mean stomach eosinophil level of 7.75 ± 3.62/hpf was observed in cases of FD. While the association between duodenal eosinophilia and specific dyspeptic symptoms was robust, the connection between stomach eosinophils and FD was less definitive. No significant associations were discovered between gastric eosinophil

levels and dyspepsia subgroups based on symptoms, indicating that gastric eosinophils may not have as contributory a role in functional dyspepsia as eosinophils in the duodenum.

Previous investigations have also showed that the existence of increased levels of gastric eosinophils were not associated with FD. In contrast to the well-established link with duodenal eosinophils, gastric eosinophilia, although present, may not significantly contribute to the symptoms of FD. Discerning this difference is crucial for comprehending the underlying mechanisms of FD and could potentially direct future investigations and therapeutic approaches. Hence, it may be safely deduced that duodenal eosinophils but not gastric eosinophils play a role in the development of the disease, consistent with other prior findings. [49]

One of the key findings in this study was the significantly higher prevalence of duodenal eosinophil degranulation among patients with functional dyspepsia (FD). 23.33% of FD patients exhibited eosinophil degranulation, compared to none of the control subjects ($p < 0.0001$). This suggests that degranulation, rather than just the presence of eosinophils, plays a pivotal role in FD pathogenesis. Eosinophil degranulation leads to the release of potent cytotoxic proteins such as eosinophil cationic protein (ECP), major basic protein (MBP), and eosinophil peroxidase (EPO), which can cause significant epithelial damage and contribute to chronic mucosal inflammation. The absence of degranulation in controls highlights that this phenomenon is specific to the dyspeptic group, indicating that eosinophil activation is more important than mere eosinophil count in FD symptomatology.[17]

The mean duodenal eosinophil count in patients with degranulation was significantly higher ($23.17 \pm 12.35\%$) compared to those without degranulation ($16.53 \pm 10.24\%$, $p = 0.012$). This finding emphasizes the importance of both eosinophil quantity and their activation state. The degranulating eosinophils are likely responsible for the release of

cytotoxic proteins that contribute to tissue damage, potentially explaining the more severe symptomatology observed in these patients. The study by Du et al. (2016) similarly reported that eosinophil degranulation is a major contributor to inflammation in eosinophilic esophagitis, a condition that shares some pathophysiological [60]

Interestingly, there was no significant difference in the duration of dyspepsia between the two groups (mean duration: 3.8 ± 2.63 years in those with degranulation vs. 4.41 ± 3.61 years in those without, p = 0.471). This suggests that degranulation is not necessarily associated with the chronicity of the disease but might instead reflect a particular phenotype of FD with more active eosinophilic inflammation. Although no significant associations were found with epigastric pain syndrome (EPS) or overlap, a significant relationship was observed between degranulation and postprandial distress syndrome (PDS) with mean symptom score of 7.53 ± 0.77 in patients with degranulation vs. 6.71 ± 1.32 in those without, p = 0.042). This highlights that degranulation might particularly affect patients with PDS subgroup of FD. These findings suggest that eosinophil degranulation could contribute to the more inflammatory forms of FD, particularly those with upper gastrointestinal discomfort.

Patients with degranulation had significantly higher SFNDI QOL scores (mean = 32.66 ± 9.08) compared to those without degranulation (mean = 27.53 ± 7.78, p = 0.001). This indicates that degranulation is associated with more severe and frequent dyspeptic symptoms, further reinforcing the role of eosinophil activation in the pathogenesis of FD. These findings are consistent with others, which reported that patients with eosinophil-mediated inflammation experience more severe symptoms, suggesting that degranulation leads to an amplified inflammatory response.[60,77,80,85]Patients with degranulation had more severe symptomatology across several measures, reinforcing the concept that activated eosinophils are more harmful than non-degranulating eosinophils. The findings highlight that targeted

therapies aimed at reducing eosinophil activation, such as corticosteroids or biologics, could be beneficial in patients with FD who exhibit eosinophil degranulation.

Overall, the findings collectively emphasize that duodenal eosinophil degranulation is not only more prevalent in FD patients but is also associated with more severe symptoms, particularly PDS subgroup. These results align with the broader literature, where eosinophil degranulation is implicated in other eosinophilic gastrointestinal disorders, such as eosinophilic esophagitis. Therefore, eosinophil degranulation could serve as a potential biomarker for identifying FD patients who may benefit from anti-inflammatory treatments targeting eosinophil activity.

The examination of gastric eosinophils showed a less significant impact on the manifestation of FD symptoms in comparison to duodenal eosinophils. The average gastric eosinophil count in patients with FD was 7.75% ± 3.62%, and there were no notable associations seen with most dyspeptic symptoms. This result is consistent with other research that has mostly concentrated on duodenal eosinophils rather than stomach eosinophils as the main location of inflammation in FD. Studies conducted by Talley et al. (2008) have demonstrated that although stomach eosinophilia can be present in FD, its occurrence does not invariably correspond to the severity of symptoms.[50] Our data, similar to most other publications, indicates that in the wider community with FD, gastric eosinophils may not play as significant a role in causing symptoms as duodenal eosinophils. Significant implications for the clinical management of FD arise from the findings of this investigation. The recognition of duodenal eosinophilia as an indicator of more advanced FD, especially in individuals experiencing retrosternal discomfort, implies that addressing inflammation caused by eosinophils could be a feasible treatment approach.

This study has established a reliable threshold for duodenal eosinophils, which physicians may utilize to categorize individuals with FD and customize therapies accordingly. Patients with eosinophil counts over the 12/hpf threshold may find alternative therapy such as early anti-inflammatory treatment advantageous, but those with lower counts may be treated with conventional therapies targeting motility or acid suppression.

The present analysis elucidates various domains that warrant further investigation. In order to determine whether duodenal eosinophilia occurs before the onset of symptoms related to FD or if it is a result of persistent dyspepsia, longitudinal investigations are necessary. Gaining insight into the chronological correlation between eosinophilia and the origin of symptoms could enhance the precision of treatment approaches and maybe halt the advancement of FD.

Furthermore, it is recommended that future research investigate the wider immunological environment in FD, namely focusing on the involvement of additional inflammatory cells such as mast cells, which have been associated with gastrointestinal problems such as irritable bowel syndrome (IBS). Conducting comparative studies between functional dyspepsia (FD) and other functional gastrointestinal illnesses could clarify both common and unique pathophysiological pathways, which may facilitate the development of more efficient and focused treatments.

However, it is necessary to conduct further research on the regional and ethnic differences in eosinophil counts and their correlation with FD. Cross-sectional studies in other populations could assist in ascertaining the general applicability of the cut-off values established in this study or the necessity for adjustments to accommodate different demographic groupings.

Ultimately, this study presents strong statistical support for the involvement of duodenal eosinophilia in functional dyspepsia, especially in subgroups that exhibit symptoms of retrosternal discomfort. Despite the variety of FD and the necessity for a tailored approach to its diagnosis and treatment, the findings are consistent with prior research. The established eosinophil cut-off provides a useful tool in the clinical toolkit, enabling the development of more individualized treatment approaches in FD. Future research should persist in investigating the intricate interaction between immune responses and gastrointestinal function, aiming to enhance outcomes for individuals with FD.

SUMMARY & CONCLUSION

SUMMARY

This prospective case-control study investigated the association between functional dyspepsia (FD) and duodenal eosinophilia in a sample of 150 FD patients and 50 controls. The study sought to identify patterns of duodenal eosinophil counts in relation to dyspeptic symptoms, demographic characteristics, and potential comorbidities. A key goal was to define a validated duodenal eosinophil cut-off point for the healthy population, which would help to define the FD patients with raised duodenal eosinophilia. The findings provide significant insights into the potential role of eosinophils in the pathogenesis of FD, particularly in patients exhibiting more severe symptoms, such as PDS and retrosternal discomfort.

The study population was well-matched in terms of age and gender distribution between FD cases and controls, eliminating the potential for demographic confounding. The mean age of FD patients was 40.88 years, slightly lower than the control group, but this difference was not statistically significant ($p = 0.531$). Similarly, gender distribution was comparable between the groups, with females slightly more prevalent in the FD group (53.33%) compared to controls (42%), but this difference was also non-significant ($p = 0.165$). Comorbidities such as hypertension, diabetes mellitus, and asthma were similarly distributed between the two groups.

The baseline SFNDI QOL score in FD patients had a mean value of 28.73 ± 8.36, reflecting the severity and frequency of dyspeptic symptoms in the study population. Additionally, the duration of dyspepsia had a mean of 4.27 ± 3.4 years, underscoring the chronic nature of the condition. Proton pump inhibitor (PPI) use, another baseline characteristic, was common among FD patients, with a mean duration of 3.61 ± 2.6 years. These findings highlight the long-term

nature of symptom management in FD and the widespread use of PPIs in treating dyspeptic symptoms.

One of the most critical findings of this study was the significantly higher duodenal eosinophil count in FD patients compared to controls. The mean duodenal eosinophil count in FD cases was 18.08 ± 11.09/hpf, compared to 8.6 ± 7.66/hpf in controls, with this difference being highly statistically significant ($p < 0.0001$).

Additionally, this study established a clinically significant duodenal eosinophil cut-off of >12/HPF in the healthy population. The ROC curve analysis demonstrated an area under the curve (AUC) of 0.749, indicating good discriminatory power. This cut-off provides a practical diagnostic threshold for identifying patients with likely FD based on eosinophil counts, which could be valuable in clinical settings for guiding treatment decisions. Using this cut-off, raised duodenal eosinophils were seen in 64.67% of enrolled FD patients.

Subgroup analysis of FD patients revealed a particularly strong association between raised eosinophil counts and specific dyspeptic symptoms. A statistically significant difference was seen in PDS and overlap symptom scores between patients with elevated eosinophil counts (mean = 6.5 ± 1.45 and 13.47 ± 1.46)/hpf and those with normal counts (mean = 7.36 ± 0.99 and 12.33 ± 1.41)/hpf with $p < 0.002$ and 0.009 respectively.

Patients with raised eosinophils had a higher prevalence of retrosternal discomfort (mean = 3.91 ± 2.19) compared to those with normal counts (mean = 3.04 ± 1.75, $p = 0.009$). These findings suggest that patients with higher duodenal eosinophil counts may represent a subtype of FD, characterized by predominantly PDS and retrosternal discomfort.

Interestingly, there was significant correlation between eosinophil counts and postprandial distress syndrome (PDS) but not epigastric

pain syndrome (EPS). This suggests that duodenal eosinophilia may not contribute to all subtypes of FD, but instead, may be more relevant to patients with PDS and retrosternal discomfort.

Duodenal eosinophil degranulation was significantly more prevalent in functional dyspepsia (FD) patients (23.33%) compared to controls (0%), indicating that eosinophil activation, rather than just eosinophil presence, plays a crucial role in FD. Patients with degranulation had higher duodenal eosinophil counts (mean 23.17 $\pm$ 12.35)/hpf compared to those without degranulation (mean 16.53 $\pm$ 10.24, $p = 0.012$), suggesting that degranulation is associated with increased eosinophilic activity. Degranulation was not correlated with the duration of dyspepsia ($p = 0.471$), but it was significantly associated with symptoms like PDS subtype ($p=0.042$) and higher overall symptom burden, as reflected in higher SFNDI QOL scores ($p = 0.001$). Overall, these results highlight degranulation as a marker of disease severity in FD and suggest that targeting eosinophil activation could be a therapeutic strategy for patients with high symptom burden.

In contrast to the strong association between duodenal eosinophilia and FD, the role of gastric eosinophils appeared to be less pronounced. The mean gastric eosinophil count in FD patients was 7.75% ± 3.62%, and there were no significant correlations between gastric eosinophils and the majority of dyspeptic symptoms.

CONCLUSION

This is the first study from India that highlights the role of duodenal eosinophilia in Indian Functional Dyspepsia patients. Our study provides a validated duodenal eosinophil cut-off for the healthy population, which could serve as a marker for FD in a subset of patients. The current findings suggest that eosinophils may be particularly relevant to FD patients with PDS or retrosternal symptoms. Eosinophilic degranulation is seen to be associated with more severe symptoms, especially PDS and poor quality of life.

The findings of this study have important clinical implications. The validated cut-off for duodenal eosinophils could serve as a useful tool for identifying FD patients who may benefit from targeted anti-inflammatory therapies. Additionally, the strong association between eosinophils and specific symptoms, such as PDS and retrosternal discomfort, suggests that a more tailored approach to FD management may be necessary. Future research should explore whether anti-eosinophilic therapies could benefit this subset of FD patients. Finally, this study highlights the need for further research into the pathophysiology of FD, particularly the role of other immune cells, such as mast cells, and the interplay between eosinophilia and other gastrointestinal disorders. Longitudinal studies are also required to establish the temporal relationship between eosinophilia and FD, which could help clarify whether eosinophilia precedes symptom onset or is a consequence of chronic inflammation.

BIBLIOGRAPHY

1. Moayyedi P, Lacy BE, Andrews CN, Enns RA, Howden CW, Vakil N. ACG and CAG Clinical Guideline: Management of Dyspepsia. Am J Gastroenterol. 2017;112:988–1013.
2. Stanghellini V, Chan FKL, Hasler WL, Malagelada JR, Suzuki H, Tack J, et al. Gastroduodenal Disorders. Gastroenterology. 2016;150:1380–92.
3. Ford AC, Forman D, Bailey AG, Axon ATR, Moayyedi P. Initial poor quality of life and new onset of dyspepsia: results from a longitudinal 10-year follow-up study. Gut. 2007;56:321–7.
4. 4. Agreus L, Svardsudd K, Nyren O, et al. Irritable bowel syndrome and dyspepsia in the general population: overlap and lack of stability over time. Gastroenterology. 1995;109:671–680.
5. Enck P, Dubois D, Marquis P. Quality of life in patients with upper gastrointestinal symptoms: results from the Domestic/International Gastroenterology Surveillance Study (DIGEST). Scand J Gastroenterol Suppl. 1999;231:48–54.
6. Brook RA, Kleinman NL, Choung RS, Melkonian AK, Smeeding JE, Talley NJ. Functional dyspepsia impacts absenteeism and direct and indirect costs. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2010;8:498–503.
7. Butterworth AE. The eosinophil and its role in immunity to helminth infection.Curr Top Microbiol Immunol. 1977;77:127–68.
8. Friesen CA, Andre L, Garola R, Hodge C, Roberts C. Activated duodenal mucosal eosinophils in children with dyspepsia: a pilot transmission electron microscopic study. J Pediatr Gastroenterol Nutr. 2002;35:329–33.
9. Drossman DA. The functional gastrointestinal disorders and the Rome III process.Gastroenterology. 2006;130:1377–90.
10. Czura CJ, Tracey KJ. Autonomic neural regulation of immunity. J Intern Med. 2005;257:156–66.

11. Holtmann G, Goebell H, Talley J. Impaired small intestinal peristaltic reflexes and sensory thresholds are independent functional disturbances in patients with chronic unexplained dyspepsia. Am J Gastroenterol. 1996;91:485–91.
12. Haug TT, Svebak S, Wilhelmsen I, Berstad A, Ursin H. Psychological factors and somatic symptoms in functional dyspepsia. A comparison with duodenal ulcer and healthy controls. J Psychosom Res. 1994;38:281–91.
13. Wilmer A, Van Cutsem E, Andrioli A, Tack J, Coremans G, Janssens J. Ambulatory gastrojejunal manometry in severe motility-like dyspepsia: lack of correlation between dysmotility, symptoms, and gastric emptying. Gut. 1998;42:235–42.
14. Futagami S, Shindo T, Kawagoe T, Horie A, Shimpuku M, Gudis K, et al. Migration of eosinophils and CCR2-/CD68-double positive cells into the duodenal mucosa of patients with postinfectious functional dyspepsia. Am J Gastroenterol. 2010;105:1835–42.
15. Wauters L, Burns G, Ceulemans M, Walker MM, Vanuytsel T, Keely S, et al. Duodenal inflammation: an emerging target for functional dyspepsia? Expert Opin Ther Targets. 2020;24:511–23.
16. Brierley SM, Linden DR. Neuroplasticity and dysfunction after gastrointestinal inflammation. Nat Rev Gastroenterol Hepatol. 2014;11:611–27.
17. Camilleri M, Coulie B, Tack JF. Visceral hypersensitivity: facts, speculations, and challenges. Gut. 2001;48:125–31.
18. Grover M, Berumen A, Peters S, Wei T, Breen-Lyles M, Harmsen WS, et al. Intestinal chemosensitivity in irritable bowel syndrome associates with small intestinal TRPV channel expression. Aliment Pharmacol Ther. 2021;54:1179–92.
19. Cenac N, Altier C, Motta JP, d'Aldebert E, Galeano S, Zamponi GW, et al. Potentiation of TRPV4 signalling by histamine and serotonin: an important mechanism for visceral hypersensitivity. Gut. 2010;59:481–8.

20. Raap U, Wardlaw AJ. A new paradigm of eosinophil granulocytes: neuroimmune interactions. Exp Dermatol. 2008;17:731–8.
21. Friesen CA, Lin Z, Singh M, Singh V, Schurman JV, Burchell N, et al. Antral inflammatory cells, gastric emptying, and electrogastrography in pediatric functional dyspepsia. Dig Dis Sci. 2008;53:2634–40.
22. Quartero AO, de Wit NJ, Lodder AC, Numans ME, Smout AJ, Hoes AW. Disturbed solid-phase gastric emptying in functional dyspepsia: a meta-analysis. Dig Dis Sci. 1998;43:2028–33.
23. Cirillo C, Bessissow T, Desmet AS, Vanheel H, Tack J, Vanden Berghe P. Evidence for neuronal and structural changes in submucous ganglia of patients with functional dyspepsia. Am J Gastroenterol. 2015;110:1205–15.
24. Xu D, Gao J, Gillilland M, Wu X, Song I, Kao JY, et al. Rifaximin alters intestinal bacteria and prevents stress-induced gut inflammation and visceral hyperalgesia in rats. Gastroenterology. 2014;146:484-96.
25. Futagami S, Itoh T, Sakamoto C. Systematic review with meta-analysis: post-infectious functional dyspepsia. Aliment Pharmacol Ther. 2015;41:177–88.
26. Miwa H, Oshima T, Tomita T, Fukui H, Kondo T, Yamasaki T, et al. Recent understanding of the pathophysiology of functional dyspepsia: role of the duodenum as the pathogenic center. J Gastroenterol. 2019;54:305–11.
27. Jacob C, Yang PC, Darmoul D, Amadesi S, Saito T, Cottrell GS, et al. Mast cell tryptase controls paracellular permeability of the intestine. Role of protease-activated receptor 2 and beta-arrestins. J Biol Chem. 2005;280:31936–48.
28. Furuta GT, Nieuwenhuis EES, Karhausen J, Gleich G, Blumberg RS, Lee JJ, et al. Eosinophils alter colonic epithelial barrier function: role for major basic protein. Am J Physiol Gastrointest Liver Physiol. 2005;289:890-97.
29. Vanuytsel T, van Wanrooy S, Vanheel H, Vanormelingen C, Verschueren S, Houben E, et al. Psychological stress and

corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism. Gut. 2014;63:1293–9.

30. Zheng PY, Feng BS, Oluwole C, Struiksma S, Chen X, Li P, et al. Psychological stress induces eosinophils to produce corticotrophin releasing hormone in the intestine. Gut. 2009;58:1473–9.
31. Yuan HP, Li Z, Zhang Y, Li XP, Li FK, Li YQ. Anxiety and depression are associated with increased counts and degranulation of duodenal mast cells in functional dyspepsia. Int J Clin Exp Med. 2015;8:8010–4.
32. Koloski NA, Jones M, Talley NJ. Evidence that independent gut-to-brain and brain-to-gut pathways operate in the irritable bowel syndrome and functional dyspepsia: a 1-year population-based prospective study. Aliment Pharmacol Ther. 2016;44:592–600.
33. Talley, NJ. Non-ulcer dyspepsia and duodenal eosinophilia: an adult endoscopic population-based case-control study. Clin Gastroenterol Hepatol 2007;5:1175–83.
34. Walker MM, Talley NJ, Prabhakar M, Pennaneac'h CJ, Aro P, Ronkainen J, et al. Duodenal mastocytosis, eosinophilia and intraepithelial lymphocytosis as possible disease markers in the irritable bowel syndrome and functional dyspepsia. Aliment Pharmacol Ther. 2009 1;29:765–73.
35. Taki M, Oshima T, Li M, Sei H, Tozawa K, Tomita T, et al. Duodenal low-grade inflammation and expression of tight junction proteins in functional dyspepsia. Neurogastroenterol Motil. 2019;31:13576.
36. Ceulemans M, Jacobs I, Wauters L, Vanuytsel T. Immune Activation in Functional Dyspepsia: Bystander Becoming the Suspect. Front Neurosci. 2022;16:831761.
37. Powell N, Walker MM, Talley NJ. The mucosal immune system: master regulator of bidirectional gut-brain communications. Nat Rev Gastroenterol Hepatol. 2017;14:143–59.
38. Vanheel H, Vicario M, Vanuytsel T, Van Oudenhove L, Martinez C, Keita ÅV, et al. Impaired duodenal mucosal integrity and low-grade inflammation in functional dyspepsia. Gut. 2014;63:262-71.

39. Liebregts T, Adam B, Bredack C, Gururatsakul M, Pilkington KR, Brierley SM, et al. Small bowel homing T cells are associated with symptoms and delayed gastric emptying in functional dyspepsia. Am J Gastroenterol. 2011;106:1089–98.
40. Kindt S, Van Oudenhove L, Broekaert D. Immune dysfunction in patients with functional gastrointestinal disorders. Neurogastroenterol Motil 2009; 21:389–98.
41. Sheikh RA, Prindiville TP, Pecha RE, Ruebner BH. Unusual presentations of eosinophilic gastroenteritis: case series and review of literature. World J Gastroenterol. 2009;15:2156–61.
42. Yu W, Freeland DMH, Nadeau KC. Food allergy: immune mechanisms, diagnosis and immunotherapy. Nat Rev Immunol. 2016;16:751–65.
43. Feinle-Bisset C, Horowitz M. Dietary factors in functional dyspepsia. Neurogastroenterol Motil. 2006;18:608–18.
44. Hurst NR, Kendig DM, Murthy KS, Grider JR. The short chain fatty acids, butyrate and propionate, have differential effects on the motility of the guinea pig colon. Neurogastroenterol Motil. 2014;26:1586–96.
45. Sun Q, Jia Q, Song L, Duan L. Alterations in fecal short-chain fatty acids in patients with irritable bowel syndrome: A systematic review and meta-analysis. Medicine (Baltimore). 2019;98:14513.
46. Zuo XL, Li YQ, Li WJ, Guo YT, Lu XF, Li JM, et al. Alterations of food antigen-specific serum immunoglobulins G and E antibodies in patients with irritable bowel syndrome and functional dyspepsia. Clin Exp Allergy J Br Soc Allergy Clin Immunol. 2007;37:823–30.
47. Skodje GI, Sarna VK, Minelle IH, Rolfsen KL, Muir JG, Gibson PR, et al. Fructan, Rather Than Gluten, Induces Symptoms in Patients With Self-Reported Non-Celiac Gluten Sensitivity. Gastroenterology. 2018;154:529-39.
48. Friesen CA, Kearns GL, Andre L, Neustrom M, Roberts CC, Abdel-Rahman SM. Clinical efficacy and pharmacokinetics of montelukast in dyspeptic children with duodenal eosinophilia. J Pediatr Gastroenterol Nutr. 2004;38:343–51.

49. Talley NJ, Walker MM, Aro P, Ronkainen J, Storskrubb T, Hindley LA, et al. Non-ulcer dyspepsia and duodenal eosinophilia: an adult endoscopic population-based case-control study. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2007;5:1175–83.
50. Talley NJ. Functional gastrointestinal disorders as a public health problem. Neurogastroenterol Motil. 2008;1:121–9.
51. Rothenberg ME, Cohen MB. An Eosinophil Hypothesis for Functional Dyspepsia. Clin Gastroenterol Hepatol. 2007;5:1147–8.
52. Walker MM, Talley NJ. Functional gastrointestinal disorders and the potential role of eosinophils. Gastroenterol Clin North Am. 2008;37:383–95.
53. Friesen CA, Neilan NA, Schurman JV, Taylor DL, Kearns GL, Abdel-Rahman SM. Montelukast in the treatment of duodenal eosinophilia in children with dyspepsia: effect on eosinophil density and activation in relation to pharmacokinetics. BMC Gastroenterol. 2009;9:32.
54. Walker MM, Salehian SS, Murray CE, Rajendran A, Hoare JM, Negus R, et al. Implications of eosinophilia in the normal duodenal biopsy - an association with allergy and functional dyspepsia. Aliment Pharmacol Ther. 2010;31:1229–36.
55. Schurman JV, Wu YP, Grayson P, Friesen CA. A pilot study to assess the efficacy of biofeedback-assisted relaxation training as an adjunct treatment for pediatric functional dyspepsia associated with duodenal eosinophilia. J Pediatr Psychol. 2010;35:837–47.
56. Furuta GT, Liacouras CA, Collins MH, Gupta SK, Justinich C, Putnam PE, et al. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. Gastroenterology. 2007;133:1342–63.
57. Binesh F, Akhondei M, Pourmirafzali H, Rajabzadeh Y. Determination of relative frequency of eosinophils and mast cells in gastric and duodenal mucosal biopsies in adults with non-ulcer dyspepsia. J Coll Physicians Surg--Pak JCPSP. 2013;23:326–9.
58. Walker MM, Aggarwal KR, Shim LS, Bassan M, Kalantar JS, Weltman MD, et al. Duodenal eosinophilia and early satiety in

functional dyspepsia: confirmation of a positive association in an Australian cohort. J Gastroenterol Hepatol. 2014;29:474–9.

59. Wang X, Li X, Ge W, Huang J, Li G, Cong Y, et al. Quantitative evaluation of duodenal eosinophils and mast cells in adult patients with functional dyspepsia. Ann Diagn Pathol. 2015;19:50–6.
60. Du L, Shen J, Kim JJ, Yu Y, Ma L, Dai N. Increased Duodenal Eosinophil Degranulation in Patients with Functional Dyspepsia: A Prospective Study. Sci Rep. 2016;6:34305.
61. Wauters L, Nightingale S, Talley NJ, Sulaiman B, Walker MM. Functional dyspepsia is associated with duodenal eosinophilia in an Australian paediatric cohort. Aliment Pharmacol Ther. 2017;45:1358–64.
62. Chaudhari AA, Rane SR, Jadhav MV. Histomorphological Spectrum of Duodenal Pathology in Functional Dyspepsia Patients.J Clin Diagn Res JCDR. 2017;11:1–4.
63. Silva J, Canão P, Espinheira MC, Trindade E, Carneiro F, Dias JA. Eosinophils in the gastrointestinal tract: how much is normal? Virchows Arch. 2018;473:313–20.
64. Sarkar AM, Akhter S, Khan MR, Saha M, Alam MR, Ghosh CK, et al. Evaluation of Duodenal Eosinophil Count in Adult Patients with Functional Dyspepsia. Mymensingh Med J MMJ. 2019;28:150–6.
65. Lúquez Mindiola A, Otero Regino W, Gómez Zuleta M. [Duodenal eosinophilia in functional dyspepsia in a Colombian sample: a case-control study]. Rev Gastroenterol Peru Organo Of Soc Gastroenterol Peru. 2019;39:21–6.
66. Burns G, Carroll G, Mathe A, Horvat J, Foster P, Walker MM, et al. Evidence for Local and Systemic Immune Activation in Functional Dyspepsia and the Irritable Bowel Syndrome: A Systematic Review. Am J Gastroenterol. 2019;114:429–36.
67. Ronkainen J, Aro P, Walker MM, Agréus L, Johansson SE, Jones M, et al. Duodenal eosinophilia is associated with functional dyspepsia and new onset gastro-oesophageal reflux disease. Aliment Pharmacol Ther. 2019;50:24–32.

68. Halland M, Talley NJ, Jones M, Murray JA, Cameron R, Walker MM. Duodenal Pathology in Patients with Rumination Syndrome: Duodenal Eosinophilia and Increased Intraepithelial Lymphocytes. Dig Dis Sci. 2019;64:832–7.
69. Leite C, Mazzoleni LE, Uchoa D de M, Castanho JA, Mazzoleni F, Sander GB. ASSOCIATION OF DUODENAL EOSINOPHILIC INFILTRATE WITH HELICOBACTER PYLORI INFECTION, BUT NOT WITH FUNCTIONAL DYSPEPSIA. Arq Gastroenterol. 2020;57:74–8.
70. Sarkar MAM, Akhter S, Khan MR, Saha M, Roy PK. Association of duodenal eosinophilia with Helicobacter pylori-negative functional dyspepsia. Arab J Gastroenterol Off Publ Pan-Arab Assoc Gastroenterol. 2020;21:19–23.
71. Potter MDE, Duncanson K, Jones MP, Walker MM, Keely S, Talley NJ. Wheat Sensitivity and Functional Dyspepsia: A Pilot, Double-Blind, Randomized, Placebo-Controlled Dietary Crossover Trial with Novel Challenge Protocol. Nutrients. 2020;12:1947.
72. Nojkov B, Zhou SY, Dolan RD, Davis EM, Appelman HD, Guo X, et al. Evidence of Duodenal Epithelial Barrier Impairment and Increased Pyroptosis in Patients With Functional Dyspepsia on Confocal Laser Endomicroscopy and “Ex Vivo” Mucosa Analysis. Off J Am Coll Gastroenterol ACG. 2020;115:1891.
73. Ronkainen J, Aro P, Jones M, Walker MM, Agréus L, Andreasson A, et al. Duodenal eosinophilia and the link to anxiety: A population-based endoscopic study. Neurogastroenterol Motil. 2021;33:14109.
74. Chehade M, Kamboj AP, Atkins D, Gehman LT. Diagnostic Delay in Patients with Eosinophilic Gastritis and/or Duodenitis: A Population-Based Study. J Allergy Clin Immunol Pract. 2021;9:2050-2059.e20.
75. Wauters L, Ceulemans M, Frings D, Lambaerts M, Accarie A, Toth J, et al. Proton Pump Inhibitors Reduce Duodenal Eosinophilia, Mast Cells, and Permeability in Patients With Functional Dyspepsia. Gastroenterology. 2021;160:1521-31.
76. Dellon ES, Gonsalves N, Rothenberg ME, Hirano I, Chehade M, Peterson KA, et al. Determination of Biopsy Yield That Optimally

Detects Eosinophilic Gastritis and/or Duodenitis in a Randomized Trial of Lirentelimab. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2022;20:535-45.

77. Shah A, Fairlie T, Brown G, Jones MP, Eslick GD, Duncanson K, et al. Duodenal Eosinophils and Mast Cells in Functional Dyspepsia: A Systematic Review and Meta-Analysis of Case-Control Studies. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2022;20:2229-42.
78. Hayatbakhsh Abbasi MM, Jafari E, Zahedi M, Darvish Moghaddam S, Taghizadeh A, Kharazmi N. Differences in Duodenal Mast Cell and Eosinophil Counts Between Patients With Functional Dyspepsia and Healthy People. Middle East J Dig Dis. 2021;13:333–8.
79. Min YW, Lee H, Ahn S, Song KH, Park JK, Shin CM, et al. Eosinophil and Mast Cell Counts in the Stomach and Duodenum of Patients with Functional Dyspepsia without a Helicobacter pylori infection. Korean J Gastroenterol Taehan Sohwagi Hakhoe Chi. 2022;80:28–33.
80. Järbrink-Sehgal ME, Sparkman J, Damron A, Walker MM, Green LK, Rosen DG, et al. Functional Dyspepsia and Duodenal Eosinophil Count and Degranulation: A Multiethnic US Veteran Cohort Study. Dig Dis Sci. 2021;66:3482–9.
81. Mahendra Raj S, Ravindran S, Kaur M, Braganza MC, Kunnath AP. Duodenal eosinophilia is associated with symptomatic erosive gastro-oesophageal reflux disease, presence of co-morbidities, and ethnicity but not undifferentiated functional dyspepsia: A retrospective Malaysian study. Med J Malaysia. 2022;77:494–9.
82. Barreyro FJ, Caronia MV, Elizondo K, Sanchez N, Jordá G, Schneider A, et al. Clinical Implications of Low-grade Duodenal Eosinophilia in Functional Dyspepsia: A Prospective Real-life Study. J Clin Gastroenterol. 2023;57:362–9.
83. Barreyro FJ, Maiorana F, Caronia MV, Elizondo K, Schneider A, Zapata PD. Association between genetic polymorphisms of NOD1, Interleukin-1B, and cagA strain with low-grade duodenal

eosinophilia in Helicobacter pylori-related dyspepsia. Helicobacter. 2023;28:13002.

84. Barreyro FJ, Sanchez N, Caronia MV, Elizondo K, Jordá G, Schneider A, et al. Low-grade duodenal eosinophilia is associated with cagA in Helicobacter pylori-related dyspepsia. J Gastroenterol Hepatol. 2023;38:274–82.
85. Huang Q, Yuan H, Li Q, Li Y, Geng S, Zhu Y, et al. Global trends in research related to functional dyspepsia and anxiety or depression over the past two decades: a bibliometric analysis. Front Neurosci. 2023;17:1218001.
86. Dellon ES, Bortey E, Chang AT, Paterson CA, Turner K, Genta RM. Determination of Optimal Eosinophil Thresholds for Diagnosis of Eosinophilic Gastritis and Duodenitis: A Pooled Analysis of 4 Prospective Studies. Clin Transl Gastroenterol. 2024;15:656.
87. Ceulemans M, Huyghe P, De Hertogh G, Cameron R, Schol J, Burns GL, et al. Redefining Histological Cell Counts Using a Standardized Method: The Leuven Intestinal Counting Protocol. Clin Transl Gastroenterol. 2024;15:725.
88. Irani MZ, Eslick GD, Burns GL, Potter M, Halland M, Keely S, et al. Coeliac disease is a strong risk factor for Gastro-oesophageal reflux disease while a gluten free diet is protective: a systematic review and meta-analysis. EClinicalMedicine. 2024;71:102577.
89. Friesen CS, Shakhnovich V, Toren P, Retke B, Schurman J, Colombo J, et al. A Pilot Study of Ketotifen in Patients Aged 8-17 Years with Functional Dyspepsia Associated with Mucosal Eosinophilia. Paediatr Drugs. 2024;26:451–7.
90. Dalal S, Ajit D, Friesen CS, Shakhnovich V, Singh M, Colombo JM, et al. Metabolomic comparison of postprandial distress syndrome patients with and without duodenal eosinophilia. J Pediatr Gastroenterol Nutr. 2024.

PROFORMA

S. No.

A. PATIENTS PARTICULARS

Name:

Age:

Sex:

Religion:

Occupation:

Address:

MRD:

Telephone no (if any):

Date of Examination:

B. CHIEF COMPLAINTS

C. HISTORY OF PRESENT ILLNESS

D. HISTORY OF PAST ILLNESS

Any GI surgery

Peptic ulcer disease

Malignancy

E. PERSONAL HISTORY

1. Smoking
2. Alcohol
3. Opium
4. Injectable drug use
5. Others

F. FAMILY HISTORY, if any

G. GENERAL SURVEY:

1. Weight
2. Height
3. BMI
4. BP
5. Others

H. LABORATORY INVESTIGATION

1) CBC
2) Absolute eosinophil count
3) RBS/FBS
4) RFT
5) TSH
6) LFT
7) ESR
8) CRP
9) ULTRASOUND ABDOMEN
10) ECG
11) CHEST XRAY
12) Stool for H. Pylori

I. ESOPHAGOGASTRODUODENOSCOPY and RUT

J. DUODENAL BIOPSY

K. SHORT FORM NEPEAN DYSPEPSIA INDEX

KEY TO MASTER CHART

SERIAL NO.	VARIABLE	REPRESENT
1	Name	Name of the Patient
2	Gender	Gender of the Patient
3	Age	Age of the Patient in years
4	Dyspepsia(yrs)	Duration of Dyspepsia in years
5	EPS	Epigastric pain syndrome subset of functional dyspepsia symptom severity score (out of 9)
6	PDS	Postprandial distress syndrome subset of functional dyspepsia symptom severity score (out of 9)
7	OVERLAP	Overlap subset of functional dyspepsia patients symptom severity score (out of 18)
8	BLOATING	Bloating severity score of functional dyspepsia patients (out of 9)
9	RETROSTERNAL DISCOMFORT	Retrosternal discomfort severity score of functional dyspepsia patients (out of 9)
10	SFNDI	Napean dyspepsia index – short form quality of life score (out of 50)
11	GASTRIC EOSINOPHILS	No of eosinophils in gastric biopsy per high power field
12	DUODENAL EOSINOPHILS	No of eosinophils in duodenal biopsy per high power field
13	DEGRANULATION	Eosinophilic degranulation present of absent in duodenal biopsy 0= absent, 1=present
14	BMI	Body mass index of patients calculated in Kg/m^2
15	HTN	Hypertension 0= absent, 1=present
16	DM	Diabetes mellitus 0= absent, 1=present
17	ASTHMA	Bronchial asthma

		0= absent, 1=present
18	TCA	History of tricyclic antidepressants use 0= absent, 1=present/received
19	SSRI	History of selective serotonin reuptake inhibitor use 0= absent, 1=present/received
20	PPI USE	History of proton pump inhibitor use 0= absent, 1=present/received
21	PPI DURATION	Duration of use of proton pump inhibitors in years

S.NO	NAME	MRD	gender	Age	dyspepsia (yrs)	EPS	bloating	Retrosternal discomfort	SFNDI	duodenal eosinophil	gastric eosinophils	degranulation	htn	DM	Asthma	PPI use	PPI duration	SSRI	TCA	BMI
1	SP	1472112	F	27	10	7	2	3	38	28	0	0	0	1	0	1	10	0	0	28
2	SK	1488321	F	41	5	6	1	1	42	12	0	0	0	0	0	1	5	0	0	22.7
3	SK	1485950	F	71	4	9	1	2	28	26	0	0	0	0	0	1	4	0	0	25
4	RKB	1475343	F	62	1	6	2	2	18	28	0	1	0	0	0	1	1	0	0	25
5	SK	1480629	F	30	2	5	2	1	19	4	0	0	0	1	0	1	2	0	0	22.8
6	MK	1484608	F	65	3	6	1	2	23	22	0	1	0	0	0	1	3	0	0	32
7	CD	1480915	F	46	2	8	2	4	22	6	0	0	0	0	0	1	1	0	0	28
8	GS	1520061	M	41	7	7	1	3	28	24	0	0	1	0	0	1	3	0	0	26.5
9	NK	1501534	F	23	4	8	3	2	20	4	0	0	1	0	0	1	4	0	0	23.7
10	SS	1427619	M	24	2	8	1	1	24	16	0	0	0	0	0	1	2	0	0	27.2
11	GS	1530136	M	30	6	9	1	1	25	6	0	0	0	1	0	1	6	0	0	24
12	VK	1534035	F	38	8	7	3	3	40	24	0	0	1	0	0	1	7	0	0	23.3
13	SA	1535064	M	14	7	8	2	4	24	8	0	0	0	0	0	1	6	0	0	29.5
14	AK	1528271	F	23	5	7	1	7	47	50	4	1	0	0	0	1	5	0	0	21.8
15	S	1540893	M	77	1	7	3	3	32	6	0	0	0	0	0	1	1	0	0	24.5
16	AG	1527379	F	18	2	8	2	6	41	10	0	1	1	0	0	1	2	0	0	23.8
17	MD	1443212	F	41	1	6	2	2	16	0	0	0	0	0	0	1	1	0	0	25.1
18	P	1499579	F	26	1	7	2	3	19	14	0	0	0	1	0	1	1	0	0	24.9
19	CS	1528185	M	37	3	8	1	3	21	2	0	0	0	0	0	1	3	0	0	22
20	AD	1675299	M	28	7	9	3	4	27	4	0	1	0	0	0	1	5	0	0	26.2
21	KK	1674658	M	49	8	7	1	3	38	22	0	1	0	0	0	1	7	0	0	22.8
22	RK	1675205	F	40	10	7	1	6	20	2	0	0	0	0	0	1	6	0	0	26.7
23	LS	1675216	M	35	2	8	2	6	16	24	0	0	0	0	0	1	2	0	0	22.3
24	RS	1647927	M	28	6	9	1	4	19	8	0	0	0	0	0	1	6	0	0	28.7
25	ND	1677997	F	46	4	8	0	6	34	22	0	0	0	0	0	1	4	0	0	24.2
26	A	1628503	M	25	6	7	1	9	28	33	0	1	0	0	0	1	6	0	0	21.53
27	ES	1682872	M	8	8	6	1	3	16	3	0	0	0	0	0	1	8	0	0	26.5
28	BK	1675099	M	32	7	8	2	2	28	25	0	1	0	0	1	1	7	0	1	21
29	SD	1683849	F	60	3	7	4	4	30	25	0	0	1	1	0	1	3	0	0	27
30	PS	1620330	F	43	11	7	3	4	26	6	0	0	0	0	0	1	10	0	0	22.4
31	TG	1692506	M	24	3	8	3	3	16	3	0	0	0	0	0	1	3	0	0	17.3
32	AS	1693736	M	23	25	7	5	2	22	15	0	0	1	0	0	1	10	0	0	28
33	MR	1693442	M	33	4	8	2	2	43	60	0	1	0	0	0	1	4	0	0	20.4
34	PS	1693542	M	53	8	7	1	1	39	29	0	0	0	1	0	1	8	0	0	28
35	PL	1448174	M	65	3	9	3	4	41	22	0	0	0	0	0	1	3	0	0	26
36	RD	1529637	F	41	2	6	2	5	28	20	0	0	0	0	0	1	2	0	0	19
37	HS	1701195	M	26	2	6	3	5	32	22	0	1	1	1	0	1	2	1	0	21.9
38	DR	1166362	F	19	1	8	3	6	26	28	0	1	0	0	0	1	1	0	0	20
39	GS	1701032	M	33	1	9	4	5	30	27	0	0	0	0	0	1	1	0	0	20.1
40	SK	1639872	F	34	4	7	3	3	18	20	0	0	0	0	0	1	1	0	0	30
41	JS	1661307	M	22	2	7	2	4	17	24	0	1	0	0	0	1	1	0	0	36
42	HS	14939	M	**40**	3	8	1	4	21	29	0	0	0	0	0	1	3	0	0	19.5
43	PK	1665621	M	59	3	7	1	5	35	28	0	0	0	1	0	1	3	0	0	19.2
44	KS	1219230	M	56	4	8	3	7	23	27	0	0	0	0	0	1	4	0	0	21.5
45	SK	14674	M	**40**	2	7	6	2	38	26	0	0	0	0	0	1	2	0	0	22
46	AK	14548	F	27	4	8	1	3	27	28	0	0	0	0	0	1	4	0	0	20.3
47	BK	1650793	F	52	1	7	1	1	19	26	0	0	0	0	0	1	1	0	0	22
48	A	159190	F	15	1	7	2	7	33	28	0	0	0	0	0	1	1	0	0	25
49	SD	1766922	f	62	1	7	4	8	35	24	12	0	0	0	0	1	1	0	0	20.5
50	BS	1240546	m	54	10	8	3	6	30	28	0	0	0	0	0	1	8	0	0	23.3
51	M	1298788	f	34	3	8	5	7	33	19	0	0	0	0	0	1	3	0	0	23
52	D	1772749	m	27	2	9	2	4	18	5	0	0	0	0	0	1	2	0	0	25

S No.	MRD	Initials	gender	Age	dyspepsia (yrs)	OVERLAP	bloating	Retrosternal discomfort	SFNDI	gastric eosinophils	duodenal eosi	degranulation	htn	DM	Asthma	PPI use	PPI duration	SSRI	TCA	BMI
1	23905	sv	F	45	7	13	4	3	31	0	12	0	0	0	0	1	2	0	0	23
2	1485269	kr	F	68	3	11	2	2	16	0	14	1	0	0	0	1	2	0	0	24.2
3	1473489	gb	M	33	4	12	1	2	14	0	28	0	0	0	0	1	3	0	1	22
4	1502764	kk	F	27	2	12	2	2	34	0	21	0	0	0	0	1	2	0	0	21.7
5	1511283	js	M	18	4	13	2	3	19	0	2	0	0	0	0	1	4	0	0	24
6	1477985	ss	M	61	2	12	3	3	15	0	8	0	0	0	0	1	1	0	0	24
7	1514405	sr	F	69	1	11	6	3	20	0	16	0	0	0	0	1	1	0	0	21.3
8	50683	ns	M	29	5	13	4	3	31	0	22	0	0	0	0	1	5	0	0	18
9	1514996	js	F	42	7	15	4	3	27	0	6	0	0	0	0	1	2	0	0	17
10	1519226	cv	M	21	8	13	2	2	28	0	16	0	0	0	0	1	3	1	0	26
11	1504202	gk	F	27	10	12	4	1	23	0	22	0	0	0	0	1	10	0	0	22
12	1042458	l	F	70	3	12	3	1	31	0	14	0	0	0	0	1	3	0	0	21.3
13	1531745	ad	F	11	4	13	5	1	36	0	20	1	0	0	0	1	4	0	0	21.8
14	1533943	js	M	73	13	15	3	6	41	0	20	1	0	1	0	1	5	0	0	19.5
15	541326	rs	M	40	2	15	2	2	25	0	30	1	0	0	0	1	1	0	0	25
16	1538020	bk	F	40	1	17	1	1	34	0	6	0	0	0	0	1	1	0	0	23.2
17	1272525	pr	F	36	5	13	2	2	24	0	23	0	0	0	0	1	5	0	0	20.5
18	1528106	jk	F	39	7	12	3	1	12	0	24	0	0	0	0	1	7	0	0	29
19	643765	pk	F	40	3	13	3	3	27	0	8	0	0	0	0	1	3	0	0	26.5
20	1674200	rg	M	51	5	12	2	1	18	0	28	0	1	1	0	1	5	0	0	24.5
21	1678286	rm	F	57	8	14	0	7	36	0	0	0	0	0	0	1	8	1	0	23.9
22	580611	kk	F	36	2	10	1	6	44	0	36	0	0	1	0	1	2	0	0	21.5
23	598235	n	F	60	5	10	3	5	37	0	26	0	0	0	0	1	5	0	0	23.4
24	1682521	ma	M	49	1	13	2	5	37	0	4	0	0	0	0	1	1	0	0	21.7
25	1668081	sd	F	78	5	14	1	4	26	0	8	0	0	0	0	1	5	0	0	24
26	1682806	kr	F	62	3	14	1	3	28	0	1	0	0	0	0	1	3	0	0	24.7
27	1676081	bk	F	54	8	11	3	3	45	0	14	1	1	1	0	1	8	0	0	23.6
28	1687719	rk	F	25	8	11	6	4	27	0	18	0	0	0	0	1	6	0	0	21.2
29	1672582	rk	F	33	5	11	7	8	37	0	23	1	0	0	0	1	5	0	0	26
30	988755	hs	M	68	7	13	8	2	29	0	26	0	0	0	0	1	7	0	0	23.3
31	1692728	pk	F	35	4	11	4	5	31	0	2	1	0	0	0	1	4	0	0	25.3
32	1579415	hs	M	61	2	13	4	7	37	0	25	0	0	0	0	1	2	0	0	23
33	1701147	ar	F	59	3	13	4	4	23	0	15	0	1	0	0	1	3	0	0	27.3
34	1494895	mj	F	50	2	13	1	4	25	0	6	0	0	0	0	1	2	0	0	26
35	1668501	m	F	27	3	12	2	3	33	0	24	0	0	1	0	1	3	0	0	33
36	1665444	k	M	48	2	15	1	2	17	0	8	0	0	0	0	1	1	0	0	27.6
37	14985	sa	F	**40**	4	14	3	7	46	0	10	1	1	0	0	1	2	0	1	22
38	1666716	t	F	20	2	15	2	2	27	0	17	0	0	0	0	1	1	0	0	24.2
39	1665196	ss	F	**40**	3	13	3	9	39	0	6	0	0	0	0	1	3	0	0	23.1
40	138960	mk	F	44	2	14	2	8	31	0	3	0	1	0	0	1	2	0	0	22.8
41	1663366	is	M	62	5	15	3	2	24	0	31	0	0	0	0	1	3	0	0	25
42	1663338	nr	F	62	3	11	6	3	16	0	13	0	0	0	0	1	2	0	0	21.3
43	1277023	a	M	32	5	11	5	4	31	0	28	0	0	1	0	1	5	1	0	20.1
44	1642425	kk	F	40	1	13	2	1	22	0	24	1	0	0	0	1	1	0	0	21.7
45	1654932	s	F	26	5	11	3	8	29	12	14	0	0	0	0	1	5	0	0	22.3
46	1655409	nk	F	19	3	13	6	7	41	0	30	1	0	0	0	1	3	0	0	21.2
47	1652388	ps	M	30	2	11	2	1	27	0	44	0	0	0	0	1	2	0	0	23.5
48	1649840	kk	F	32	1	13	2	3	17	0	26	0	0	1	0	1	1	0	0	23
49	7265	gk	f	16	1	11	8	2	27	0	24	0	0	0	0	1	1	0	0	21.4
50	107153	ks	m	29	4	15	2	4	27	0	18	0	0	0	0	1	4	0	0	22.5
51	1766673	mk	f	34	4	12	3	2	28	0	22	0	0	0	0	1	4	0	0	24
52	1760233	sk	f	35	3	13	1	2	45	0	22	0	0	0	0	1	3	0	0	25
53	1781074	pk	f	50	8	11	3	5	36	8	3	0	0	0	0	1	6	0	0	27

S.No.	MRD	Initials	gender	Age	dyspepsia(yrs)	PDS	bloating	Retrosternal discomfort	SFNDI	duodenal eosinophil	gastric eosinophils	degranulation	htn	DM	Asthma	PPI use	PPI duration	SSRI	TCA	BMI
1	1486680	ak	F	57	6	8	2	1	29	22	0	0	1	0	1	1	3	1	0	21.8
2	1485958	rr	F	22	20	7	1	4	35	16	0	0	0	0	0	1	18	0	0	22.3
3	1479628	bs	M	61	1	4	5	3	12	6	0	0	0	0	0	1	1	0	0	25.7
4	1477050	rs	M	38	12	9	6	3	27	26	0	0	0	0	0	1	10	0	0	28.9
5	1486660	rb	M	17	5	7	4	3	38	2	0	0	0	0	0	1	3	0	0	25.3
6	595885	pk	F	77	3	4	3	3	30	4	0	0	0	1	0	1	3	0	0	25.1
7	1517899	mk	M	18	3	6	4	1	17	4	0	0	1	0	0	1	3	0	0	22.2
8	335278	as	M	42	5	7	5	2	21	6	0	0	0	0	0	1	5	0	0	27.3
9	1462127	mp	F	29	4	7	2	6	43	16	0	1	0	0	0	1	4	0	0	25.3
10	1466262	r	M	17	2	6	4	2	30	32	0	1	0	0	0	1	2	0	0	26.1
11	1530683	ag	F	44	7	4	7	2	22	12	0	1	0	1	0	1	4	0	0	28.5
12	1008458	s	M	29	5	6	7	2	27	26	10	1	0	0	0	1	2	0	0	23.4
13	1532094	ik	F	62	3	8	6	2	28	20	0	1	0	0	0	1	3	0	1	23.3
14	1539886	ks	F	68	4	7	3	1	20	12	0	0	0	0	0	1	4	0	0	27
15	130404	rk	F	**40**	1	6	4	2	26	20	0	1	0	0	0	1	1	0	0	25
16	1539207	mk	M	**40**	1	6	3	1	28	8	0	0	0	0	0	1	1	0	0	22
17	790951	vk	M	34	4	7	2	6	35	10	0	1	0	0	1	1	4	0	0	25.3
18	1511231	ds	M	14	5	6	1	2	18	24	0	0	0	0	0	1	3	0	0	23.2
19	1477985	ss	M	61	4	7	2	2	26	6	0	0	0	0	0	1	4	1	0	25.5
20	1671523	m	F	42	6	8	3	2	33	6	0	0	1	0	0	1	6	0	0	22.8
21	1665228	js	M	62	3	7	2	1	36	2	0	0	1	0	0	1	3	0	1	21.3
22	1666749	js	M	35	2	8	3	2	45	18	0	1	1	0	0	1	2	0	0	24
23	1680581	ss	M	98	4	8	2	3	29	5	0	1	1	0	0	1	4	0	0	26.8
24	1668307	gs	M	80	7	4	2	2	31	10	8	0	1	0	0	1	7	0	0	23.5
25	1681401	kk	F	49	1	8	3	4	33	7	0	0	0	0	0	1	1	0	0	22.1
26	1618528	as	M	40	1	8	1	8	41	30	0	1	0	0	0	1	1	0	0	26.5
27	1638091	sk	F	23	4	7	5	5	13	6	0	0	0	1	0	1	4	0	0	17.1
28	1696379	mt	F	27	2	7	3	3	31	6	0	0	0	0	0	1	1	0	0	30
29	1676897	am	M	18	1	8	5	5	38	20	0	0	1	0	1	1	1	0	0	24.3
30	1702889	nr	F	76	1	6	2	6	41	40	0	1	0	0	0	1	1	0	0	28
31	1664089	hk	F	25	3	8	2	4	24	22	0	0	0	0	0	1	3	0	0	28.5
32	1403101	rk	M	73	5	6	2	5	35	27	0	0	1	0	0	1	5	0	0	23.1
33	1665379	ck	F	50	6	8	3	4	32	35	0	0	1	0	0	1	6	0	0	25.2
34	14935	rk	M	**40**	1	8	0	4	27	25	0	0	0	0	0	1	1	0	0	19.4
35	15010	ak	M	48	2	8	2	8	26	6	0	1	0	0	0	1	2	0	0	25.8
36	1663459	ss	M	58	1	8	2	1	28	32	0	0	0	0	0	1	1	0	0	24.2
37	991247	mk	F	55	4	8	4	5	19	25	0	0	0	0	1	1	4	0	0	19.7
38	1653062	ks	M	80	3	8	5	2	37	4	0	0	1	0	0	1	3	0	0	30.5
39	1654041	k	M	**40**	8	7	4	2	32	24	2	0	0	0	0	1	8	0	0	31.6
40	1646808	rs	M	62	5	7	1	8	21	36	0	0	1	0	0	1	5	0	0	21.9
41	1518557	pj	M	12	2	9	2	3	42	20	6	1	0	0	0	1	1	0	0	28
42	1775745	jj	m	40	3	8	2	3	30	30	0	0	0	0	0	1	2	0	0	28.9
43	1272885	bj	m	38	2	6	7	7	26	32	0	0	0	0	0	1	2	0	0	22.2
44	1778342	a	f	18	1	8	2	8	41	37	0	0	0	0	0	1	1	0	0	28
45	1778411	a	f	51	12	6	2	2	42	12	0	0	0	0	0	1	8	0	0	32.8

Sr.No.	Initials	gender	Age	Dyspepsia	duodenal eosinophil	htn	DM	Asthma	SSRI	TCA
1	a	M	26	no	4	0	0	n	0	0
2	s	M	54	no	3	0	0	n	0	0
3	s	M	53	no	4	0	0	n	0	0
4	g	M	27	no	6	0	0	n	0	0
5	c	M	76	no	7	0	0	n	1	0
6	n	M	56	no	5	0	0	n	0	0
7	n	M	46	no	3	1	0	n	0	0
8	s	F	45	no	8	0	1	n	0	0
9	g	M	48	no	7	0	0	n	0	0
10	s	F	20	no	5	0	0	n	0	0
11	r	F	22	no	10	0	0	n	0	0
12	a	F	35	no	12	0	0	n	0	0
13	s	F	47	no	24	0	0	n	0	0
14	op	F	67	no	32	0	0	n	0	0
15	s	M	54	no	3	0	0	n	1	0
16	r	M	51	no	6	0	0	n	0	1
17	m	M	53	no	5	0	0	n	0	0
18	r	F	37	no	4	0	0	n	0	0
19	a	M	34	no	8	1	0	n	0	0
20	d	M	24	no	8	0	0	n	0	0
21	p	M	26	no	6	0	0	n	0	0
22	p	F	33	no	5	0	1	n	0	0
23	hs	F	41	no	4	0	0	n	0	0
24	r	F	42	no	8	0	0	n	0	0
25	r	F	28	no	16	0	0	n	1	0
26	s	F	29	no	4	0	0	n	1	0
27	b	M	31	no	6	0	0	n	0	1
28	k	M	43	no	8	0	0	n	0	0
29	a	M	24	no	8	0	0	n	0	0
30	s	M	36	no	5	0	0	n	0	0
31	**s**	F	27	no	7	0	0	n	0	0
32	k	M	38	no	22	0	0	n	0	0
33	k	F	46	no	4	0	0	n	0	0
34	n	M	45	no	6	1	0	n	0	0
35	k	F	34	no	4	1	0	n	0	0
36	s	M	29	no	3	1	0	n	1	0
37	m	F	34	no	10	1	1	n	0	0
38	s	M	49	no	0	0	1	n	0	0
39	rs	M	44	no	20	0	0	n	0	1
40	r	F	72	no	23	0	0	n	0	0
41	n	F	65	no	2	0	0	n	0	0
42	a	F	22	no	5	0	0	n	0	0
43	r	F	59	no	21	0	0	n	0	0
44	m	M	42	no	0	0	0	n	0	1
45	m	M	38	no	12	1	0	n	0	0
46	r	M	45	no	35	0	0	n	0	0
47	c	M	63	no	6	0	0	n	0	0
48	m	M	21	no	8	0	0	n	0	0
49	k	M	32	no	5	0	0	n	0	1
50	s	F	63	no	3	1	0	n	0	0

Baba Farid University
of Health Sciences
Sadiq Road
Faridkot-151 203 (Pb). INDIA.
Phone : 01639-256232,256236
Tele/Fax : 01639-256235
Email – Thesisbfuhs@gmail.com

(80)

No. BFUHS/2K23p-TH/ 70 Date 1/1/24

The Principal
Dayanand Medical College & Hospital
Ludhiana

Subject:: Approval of Plan of Thesis.

This is to inform you that the Faculty of Medical Sciences in its meeting held on 25.09.2023 has approved the plan of thesis of **Manisha Khubber**, a student of your college for **DM Medical Gastroenterology** along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis
Association of functional dyspepsia with duodenal eosinophilia: a prospective case control study .

Supervisor	Co-Supervisor:-	Co-Supervisor:-
Dr. Omesh Goyal	Dr. Ajit Sood	Dr. Harpreet Kaur
Professor	Prof. & Head	Prof. & Head
Dept. of Gastroenterology	Dept. of Gastroenterology	Dept. of Pathology

The student may submit the thesis in triplicate alongwith the plan in triplicate also, to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She may be directed to attach a photocopy of this letter with the forwarding letter while submitting thesis in the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic, if any.

Controller of Examinations

Note :

1. *If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.*

ASSOCIATION OF FUNCTIONAL DYSPEPSIA WITH DUODENAL EOSINOPHILIA: A PROSPECTIVE CASE CONTROL STUDY

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DM

IN

GASTROENTEROLOGY

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES
FARIDKOT

NOVEMBER, 2022

DR. MANISHA KHUBBER

DEPARTMENT OF GASTROENTEROLOGY
DAYANAND MEDICAL COLLEGE &HOSPITAL
LUDHIANA

ABSTRACT OF PLAN OF THESIS

Title	ASSOCIATION OF FUNCTIONAL DYSPEPSIA WITH DUODENAL EOSINOPHILIA : A PROSPECTIVE CASE CONTROL STUDY
For the degree of	DM Gastroenterology
Name of the candidate	Dr. Manisha Khubber
Supervisor	Dr. Omesh Goyal, Professor, Department of Gastroenterology, DMCH Ludhiana
Co-supervisor	Dr. Ajit Sood, Professor & Head, Department of Gastroenterology, DMCH Ludhiana Dr. Harpreet Kaur, Professor & Head, Department of Pathology, DMCH Ludhiana.
Institution	Dayanand Medical College & Hospital, Ludhiana
University	Baba Farid University of Health Sciences, Faridkot

Introduction: Functional Dyspepsia is a common clinical entity throughout the world irrespective of geographical locations and ethnicities, with a reported prevalence of 10-30% worldwide, and an annual incidence of 1–3%. The pathophysiology of FD is largely undetermined and satisfactory treatment is not available. Several studies have recently suggested a relationship between increased numbers of duodenal eosinophils with nonulcer dyspepsia. The potential role of duodenal eosinophils in the pathophysiology of Functional Dyspepsia requires further investigation. Such studies on Indian subjects especially in North Indian population are lacking. This study will focus on determining whether duodenal eosinophilia is associated with functional dyspepsia in patients presenting at our hospital.

Aim of Study: PRIMARY OBJECTIVE: To determine whether patients with functional dyspepsia have increased eosinophil count in duodenal biopsy compared to healthy controls. SECONDARY OBJECTIVE: To define normal duodenal eosinophil count in healthy North Indian population.

Materials & methods:. Total number of cases will be 50 including both males and females comprising of patients who will present with functional dyspepsia. They will be compared with duodenal biopsies 50 age and sex matched control subjects from general population of same socioeconomic status who underwent endoscopy without symptoms of dyspepsia.

Clinical Significance: To study if duodenal eosinophilia is responsible for functional dyspepsia

Keywords: Functional dyspepsia, duodenal eosinophilia.

(Signature) Candidate	(Signature) Co-supervisor	(Signature) Co-supervisor	(Signature) Supervisor	(Signature) Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"ASSOCIATION OF FUNCTIONAL DYSPEPSIA WITH DUODENAL EOSINOPHILIA: A PROSPECTIVE CASE CONTROL STUDY"** exist in the Department of Gastroenterology, DMC&H, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis is genuine and is collected by the candidate herself under our supervision and guidance.

Supervisor

Dr. Omesh Goyal
Professor
Department of Gastroenterology
DMCH, Ludhiana

Co-Supervisor

Dr. Ajit Sood
Professor & Head
Department of Gastroenterology
DMCH, Ludhiana

Dr. Harpreet Kaur
Professor & Head
Department of Pathology
DMCH, Ludhiana

Head of the Department

Dr. Ajit Sood
Professor & Head
Department of Gastroenterology
DMCH, Ludhiana

Place : _____

Date : _____

APPROVAL PROFORMA
BY RESEARCH & ETHICAL COMMITTEE
DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA

Name of candidate	Dr. Manisha Khubber
Department	DM Gastroenterology
Topic of Thesis	ASSOCIATION OF FUNCTIONAL DYSPEPSIA WITH DUODENAL EOSINOPHILIA: A PROSPECTIVE CASE CONTROL STUDY
Likely date of appearing in PG examination	May/ June, 2024
Date of enrollment	27.04.2022
Name of Head of Department	Dr. Ajit Sood
1) Supervisor **2) Co-Supervisor**	Dr. Omesh Goyal Dr. Ajit Sood Dr. Harpreet Kaur
Signature of Members of Research Committee with Stamp	**Signature of Members of Ethics Committee with Stamp**
Dr. Harpreet Kaur (Professor & Head, Pathology)	Mr. R K Sharma (Legal Expert)
Dr. Jaspal Singh (Professor & Head, Surgery)	Dr. Ramit Mahajan (Associate Professor, Gastroenterology)
Dr. Dinesh Gupta (Professor & Head, Medicine)	Dr. Anu Sharma (Professor, Anatomy)
Dr. Sandeep Kaushal (Dean Academics & Professor & Head, Pharmacology)	Dr. Sandeep Kaushal (Dean Academics & Professor & Head, Pharmacology)
Dr. Gurpreet S Wander (Vice Principal)	Dr. Gurpreet S Wander (Vice Principal & Professor & Head, Cardiology)
	Dr. Gagandeep Singh (Secretary, Ethics Committee Professor & Head, Neurology)

Approved: Yes / No

Dr. Sandeep Puri
Chairperson
Research Committee
Chairperson
Research Committee

Approved: Yes / No

Prof. Arvind Malhotra
Chairperson
Ethics Committee
Chairperson
Ethics Committee

BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT

PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

1	NAME OF THE CANDIDATE AND ADDRESS	Dr. MANISHA KHUBBER H. NO. 418P, SECTOR 8, URBAN ESTATE, AMBALA CITY, HARYANA 134003
2	NAME OF THE INSTITUTE	DAYANAND MEDICAL COLLEGE AND HOSPITAL, LUDHIANA
3	COURSE OF STUDY AND SUBJECT	D.M. MEDICAL GASTROENTEROLOGY
4	DATE OF ADMISSION TO COURSE	27-04-2022
5	TITLE OF THE TOPIC	ASSOCIATION OF FUNCTIONAL DYSPEPSIA WITH DUODENAL EOSINOPHILIA: A PROSPECTIVE CASE CONTROL STUDY

6.0 BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR STUDY

Dyspepsia is a very common presenting complaint in a large number of OPD and IPD patients presenting in a hospital. Most of the patients presenting with dyspepsia undergo an esophagogastroduodenoscopy to rule out an underlying organic cause prior to labelling a patient as functional dyspepsia. Prevalence of functional dyspepsia is very high worldwide. There is an increasing evidence of various pathophysiological mechanisms underlying FD. Lately, a large focus has been drawn towards altered gastrointestinal dysmotility, H. pylori, psychological disturbances and low grade immune activation.[1] Several studies have recently suggested a relationship between increased numbers of duodenal eosinophils with nonulcer dyspepsia, early satiety in particular, and these might contribute to gastroinetinal symptoms development and persistence. Mast cells and eosinophilic degranulation has been speculated to contribute to the aetiopathogenesis of functional dyspepsia. Eosinophils are also associated with gastric dysmotility.[2] The potential role of duodenal eosinophils in the pathophysiology of FD requires further investigation, including elucidation of mechanisms of eosinophil recruitment and activation, association with functional studies such as manometry and gastric accommodation, and evaluation of targeted therapies. However, the normal eosinophilic content of the duodenal mucosa has been investigated only sporadically. Since no consensus exists on specific limits for normality, there are no guidelines as to what constitutes an excessive number of eosinophils in the duodenum and how a diagnosis of duodenal eosinophilia can be made. Duodenal eosinophilia may potentially alter the current diagnostic approach with implications for therapy if confirmed in larger studies.

6.2 REVIEW OF LITERATURE-

Dyspepsia, in general, is a term classically used to describe symptoms referable to the upper gastrointestinal tract. The term dyspepsia was first coined in the eighteenth centuary. Alarm features of dyspepsia like anemia, dysphagia, unintended weight loss GI bleed or abdominal mass warrant investigation to exclude organic diseases like gastrointestinal neoplasias. After organic pathologies have been ruled out by esophagogastroduodenoscopy and other necessary investigations, the patient is labelled as functional dyspepsia.[1]

FD can further be subdivided into those with predominant epigastric pain (epigastric pain syndrome, EPS) or early satiety/post prandial fullness (post-prandial distress syndrome, PDS).[1] Refinement of this definition has sought to exclude patients with heartburn and regurgitation, and have culminated in the Rome IV criteria which specifically defines dyspepsia as troublesome epigastric pain or burning, early satiety or post prandial fullness within the past 3 months with symptom onset at least 6 months earlier in the absence of any organic or structural disease. Due to lack of specific biomarkers, ROME IV criteria are widely used to diagnose functional dyspepsia. [3]

FD is a common clinical entity throughout the world irrespective of geographical locations and ethnicities, with a reported prevalence of 10-30% worldwide, and an annual incidence of 1–3%. The pathophysiology of FD is largely undetermined and satisfactory treatment is not available. Hence, the patients usually undergo repeated medical examinations leading to a substantial burden on healthcare resources.[4]

Even though no association has been seen between functional dyspepsia and decreased survival, it is significantly associated with a decreased quality of life and a considerable burden to the healthcare system as well as the patients in terms of reduced opportunity costs.[5]

In general, physcians ascribe pathology to symptoms and treatment is prescribed based on the respective pathology underlying the disease. Hence functional gastrointestinal disorders are often perceived to be as difficult to treat. However, as advances are made in attempting to understand the underlying mechanisms FGIDs, there is increasing evidence suggesting a link to localized derangements in the GI-neural axis, including motor and sensory abnormalities, neuro-immuno dysregulation[6] and enhanced sensitivity to luminal contents.[7] There are few cost-effective and simple tests that can identify dysmotility in vivo and whilst endoscopy is often performed in these patients, biopsy is not routine to identify subtle pathology changes.[8]

FD represents a wide spectrum of heterogeneous conditions, however, the pathophysiological mechanisms are not very well understood. Lately, a large focus has been drawn towards altered gastro-duodenal motility, gastro-duodenal hypersensitivity in response to gastric distention, Helicobacter pylori (H. pylori) infection, and psychological disturbance. Recently, increasing evidence suggests presence of low-grade immune activation in the duodenal mucosa of funcyional dyspepsia patients.[2,9]

Holtmann et al. found significantly increased levels of systematic cytokines like IL-1β, IL-10 and TNF-α in functional dyspepsia patients in comparison with healthy subjects.[10] Furthermore, it has been hypothesized that mast cells and eosinophils are involved in the pathogenesis of FD and these might contribute to gastrointestinal symptoms development and persistence. FD patients have been seen to have increased mast cell and eosinophil infiltration in the duodenum indicating ongoing low-grade inflammation.[11]

The eosinophil are characteristically bone marrow derived circulating granulocytes having a multitude functions. In GIT eosinophils are responsible for important effector mechanisms in normal physiology throughout in healthy subjects. However, squamous epithelium of the oesophagus is normally devoid of eosinophils.[12] The normal function of the eosinophil in host defence and immune regulation is carried out via effector secretory granules, which effect antigen presentation, cytokine release, mast cell activation and immune tolerance. Although there role in helminthic infections is well established, eosinophils play an important role in viral and bacterial infections also. Eosinophilic dysregulation also occurs in drug reactions and hypersensitivity, allergic disease, parasite infestation and tumours.[13]

Mast cells and eosinophils are often co-dependent; initially, mast cells induce eosinophils to migrate into the mucosa and these in turn activate mast cells via mediators and growth factors, causing proliferation, maturation and degranulation.[14] Eosinophils are associated with gastric dysmotility.[15] The enteric immune response may be localized to the gut mucosa and may not be apparent systemically that is without a corresponding rise in serological IgE.[16]

Eosinophils and mast cells also communicate with T lymphocytes in the gut mucosa. In one report of patients with FD and normal duodenal histology, a significantly lower percentage of duodenal CD3+ IEL expressed T lymphocyte activation markers CD95 ／ Fas and human leukocyte antigen-DR (HLA-DR) in Helicobacter pylori negative patients with FD as compared to healthy controls even though the number of duodenal intraepithelial lymphocytes (IELs) were similar in both groups.[17]

Eosinophils are known to connect innate and adaptive immune systems. A shift from a Th1 to a Th2-mediated response by eosinophils has also been proposed after enteric infection or allergen exposure.[18] One study reported that the rectal biopsies in post infectious IBS patients

showed significantly increased T lymphocytes in the lamina propria and mast cells (but not IELs) compared to controls.[19]

Duodenal eosinophilia and degranulation have been recently seen in FD. Various studies have shown correlation of duodenal eosinophils with the symptoms of FD, and early satiety in particular. Eosinophilic degranulation has been speculated to contribute to the aetiopathogenesis of FD.[2]

In general, the prevalence of FD in patients with asthma and allergic rhinitis is seen to be higher than healthy populations. Duodenal eosinophilia has been observed in atopic patients. Therapeutic effect of eosinophil stabilising drug, monteleukast, in children with FD suggests a potential pathophysiological role but confirmatory studies for the same are lacking. In an Australian study in pediatric patients with FD, duodenal eosinophilia was observed in 71–95%, but it remains to be ascertained whether this represents a true pathology. As such, the significance between two entities is controversial.[20]

In a population-based case-control study, it was observed that a higher proportion of patients with FD had ≥22 eosinophil count (5 non-overlapping high powered fields, HPFs) in first portion of the duodenum compared to asymptomatic controls.[2]

However, duodenal eosinophilia was found only in post-infectious FD patients in a study conducted in Japanese patients with FD.[21] One Australian study showed significantly elevated duodenal eosinophil count but no mast cell increase in FD patients.[22] Another case-control study in Iranian FD patients failed to demonstrate any association between FD and duodenal eosinophilis and mast cells.[23] The association between FD and duodenal eosinophil and mast cell through various studies remains inconsistent. Some of these studies were limited by small sample size or had other confounding factors.

Such studies on Indian subjects especially in North Indian population are lacking. This study will focus on determining whether duodenal eosinophilia is associated with functional dyspepsia in patients presenting at our hospital.

6.3 AIMS AND OBJECTIVES-

PRIMARY OBJECTIVE: To determine whether patients with functional dyspepsia have increased eosinophil count in duodenal biopsy compared to healthy controls.

SECONDARY OBJECTIVE:

1. To define normal duodenal eosinophil count in healthy North Indian population.
2. To compare symptoms severity and quality of life using SFNDI quesionnaire between patients with normal duodenal eosinophil count and those with increased counts.

.

7.0 MATERIAL AND METHODS:

7.1 SOURCE OF DATA: The study will be an observational cross sectional comparative hospital based study and will be conducted in Department of Medical Gastroenterology, Dayanand Medical College and Hospital, Ludhiana in collaboration with department of Pathology, Dayanand Medical College and Hospital, Ludhiana, Punjab.

Study population: Patients coming to the department for esophagogastroduodenoscopy will be included in the study. Total number of cases will be 50 including both males and females comprising of patients who will present with functional dyspepsia. They will be compared with duodenal biopsies 50 age and sex matched control subjects from general population of same socioeconomic status who underwent endoscopy without symptoms of dyspepsia. Study will be conducted after approval from the institutions ethics committee.

Informed Consent will be taken from all the participants included in the study.

Group A: comprising of 50 people with functional dyspepsia

Group B: comprising of 50 age and sex matched control subjects.

The patients and controls in two groups (A&B) will be subjected to routine history taking, systemic examination, esophago-gastro-duodenoscopy and duodenal biopsy. Lab investigations will be noted.

7.2 METHOD OF COLLECTION OF DATA

INCLUSION CRITERIA (CASES)

- Age > 18 years.
- Typical history of dyspepsia meeting ROME IV criteria
- Normal esophago-gastro-duodenoscopy

INCLUSION CRITERIA (CONTROLS)

- Age >18years.
- Subjects who underwent endoscopy for indications other than dyspepsia like anemia, diarrhea or incidental biopsy.
- No history of active GI bleed.

EXCLUSION CRITERIA

- Inflammatory Bowel Disease
- History of peptic ulcer
- Any eosinophilic condition of any other area of GIT, Parasitic infections or Peripheral eosinophilia
- Any duodenal polyp, nodule, stricture or duodenitis
- Progressive severe disease requiring active medical management (CAD/ CKD/ malignancy/cirrhosis/uncontrolled diabetes or hypertension)
- Positive for H. pylori
- History of upper gastrointestinal surgery
- Drugs causing eosinophilia like antiepileptics (carbamazepine, phenytoin, lamotrigine), fluoxetine, captopril and antibiotics (ampicillin, isoniazid, rifampin, linezolid, trimethoprim-sulfmethoxazole, vancomycin)
- Drugs causing duodenal inflammation like NSAIDS, aspirin or clopidogrel.

The Short Form (SF) Nepean Dyspepsia Index is a 10-item questionnaire with 5 sub-scales each examining the influence of dyspepsia on domains of health in patients, namely tension/anxiety, interference with daily activities, disruption to regular eating/drinking, knowledge towards/control

over disease symptoms and interference with work/study, with each sub-scale containing two items. Each item is measured by a 5-point Likert scale ranging from 0 (not at all or not applicable), 1 (a little), 2 (moderately), 3 (quite a lot) to 4 (extremely). Individual items in each sub-scale are aggregated to obtain a score range from 0 (lowest HRQoL score) to 100 (highest HRQoL score) as per the developers' original calculation formula. A total, overall SF-NDI total score is obtained using the mean of 5 subscale scores.

DURATION OF THE STUDY- 1 January 2023 to 28 February 2024.

OUTCOME: To evaluate association of functional dyspepsia with duodenal eosinophilia and to compare symptoms severity and quality of life using SFNDI quesionnaire between patients with normal duodenal eosinophil count and those with increased counts.

STATISTICAL ANALYSIS:

Data were described in terms of range; mean ±standard deviation (± SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. To determine whether the data were normally distributed, a Kolmogorov-Smirnov test was used. Comparison of quantitative variables between the study groups was done using student t test and Mann Whitney test for parametric and non-parametric data respectively. For comparing categorical data, Chi square ($\chi 2$) test was performed and fisher exact test was used when the expected frequency is less than 5. A probability value (p value)less than 0.05 was considered statistically significant. All statistical calculations were done using (Statistical Package for the Social Science)SPSS 21version (SPSS Inc., Chicago, IL, USA)statistical program for Microsoft Windows.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

Yes, Routine lab investigations and upper gastrointestinal endoscopy.

7.4 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Yes, Attached herewith

8. LIST OF REFERENCES

1. Moayyedi PM, Lacy BE, Andrews CN, Enns RA, Howden CW, Vakil N. ACG and CAG clinical guideline: management of dyspepsia. Official journal of the American College of Gastroenterology| ACG. 2017;112:988-1013.
2. Talley NJ, Walker MM, Aro P, Ronkainen J, Storskrubb T, Hindley LA, et al. Non-ulcer dyspepsia and duodenal eosinophilia: an adult endoscopic population-based case-control study. Clinical Gastroenterology and Hepatology. 2007;5:1175-83.
3. Stanghellini V, Chan FK, Hasler WL, Malagelada JR, Suzuki H, Tack J, et al. Gastroduodenal disorders. Gastroenterology. 2016;150:1380-92.
4. Agréus L, Svärdsudd K, Nyrén O, Tibblin G. Irritable bowel syndrome and dyspepsia in the general population: overlap and lack of stability over time. Gastroenterology. 1995;109:671-80.
5. Brook RA, Kleinman NL, Melkonian AK, Smeeding JE, Talley NJ. Functional dyspepsia impacts absenteeism and direct and indirect costs. Clinical Gastroenterology and Hepatology. 2010;8:498-503.
6. Czura CJ, Tracey KJ. Autonomic neural regulation of immunity. J Intern Med. 2005;257:156–66
7. Holtmann G, Geobell H, Talley NJ. Impaired small intestinal peristaltic reflexes and sensory thresholds are independent functional disturbances in patients with chronic unexplained dyspepsia. Am J Gastroenterol. 1996;91:485–91.
8. Tack J, Talley NJ, Camilleri M, Holtmann G, Malagelada J, Stanghellini V. Functional gastroduodenal disorders. Gastroenterology. 2006;130:1466–79.
9. Haug TT, Svebak S, Wilhelmsen I, Berstad A, Ursin H. Psychological factors and somatic symptoms in functional dyspepsia. A comparison with duodenal ulcer and healthy controls. Journal of psychosomatic research. 1994;38:281-91.

10. Liebregts T, Adam B, Bredack C, Gururatsakul M, Pilkington KR, Brierley SM, et al. Small bowel homing T cells are associated with symptoms and delayed gastric emptying in functional dyspepsia. Official journal of the American College of Gastroenterology| ACG. 2011;106:1089-98.
11. Martínez Martínez C, Vicario Perez M, Santos Vicente FJ, Farre R. Impaired duodenal mucosal integrity and low-grade inflammation in functional dyspepsia. Gut. 2014;63:262-71.
12. Rothenberg ME, Hogan SP. The eosinophil. Annual review of immunology. 2006;24:147-74.
13. Butterworth A. The eosinophil and its role in immunity to helminth infection. Curr Top Microbiol Immunol. 1977;77:127-68.
14. Piliponsky AM, Pickholtz D, Gleich GJ, Levi-Schaffer F. Human eosinophils induce histamine release from antigen activated rat peritoneal mast cells: a possible role for mast cells in late-phase allergic reactions. J Allergy Clin Immunol. 2001;107:993-1000.
15. Martin ST, Collins CG, Fitzgibbon J, Lee G, Quigley EM, O'Sullivan GC. Gastric motor dysfunction: is eosinophilic mural gastritis a causative factor? Eur J Gastroenterol Hepatol. 2005;17:983-6.
16. Coeffier M, Lorentz A, Manns MP, Bischoff SC. Epsilon germ-line and IL-4 transcripts are expressed in human intestinal mucosa and enhanced in patients with food allergy. Allergy. 2005;60:822-7.
17. Gargala G, Lecleire S, François A, Jacquot S, Déchelotte P, Ballet JJ, et al. Duodenal intraepithelial T lymphocytes in patients with functional dyspepsia. World journal of gastroenterology: WJG. 2007;13:2333-8.
18. Kindt S, Van Oudenhove L, Broekaert D, Kasran A, Ceuppens JL, Bossuyt X, et al. Immune dysfunction in patients with functional gastrointestinal disorders. Neurogastroenterology & Motility. 2009;21:389-98.

19. Dunlop SP, Jenkins D, Spiller RC. Distinctive clinical, psychological, and histological features of postinfective irritable bowel syndrome. The American journal of gastroenterology. 2003;98:1578-83.
20. Friesen CA, Andre L, Garola R, Hodge C, Roberts C. Activated duodenal mucosal eosinophils in children with dyspepsia: a pilot transmission electron microscopic study. Journal of pediatric gastroenterology and nutrition. 2002;35:329-33.
21. Futagami S, Shindo T, Kawagoe T, Horie A, Shimpuku M, Gudis K, et al. Migration of eosinophils and CCR2-/CD68-double positive cells into the duodenal mucosa of patients with postinfectious functional dyspepsia. Official journal of the American College of Gastroenterology| ACG. 2010;105:1835-42.
22. Walker MM, Salehian SS, Murray CE, Hoare JM, Powell N, Talley NJ, et al. Implications of eosinophilia in the normal duodenal biopsy - an association with allergy and functional dyspepsia. Aliment Pharmacol Ther. 2010;31:1229-36.
23. Binesh F, Akhondei M, Pourmirafzali H, Rajabzadeh Y. Determination of relative frequency of eosinophils and mast cells in gastric and duodenal mucosal biopsies in adults with non-ulcer dyspepsia. J Coll Physicians Surg Pak. 2013;23:326-9.

PROFORMA

S. No.

A. PATIENTS PARTICULARS

Name:

Age:

Sex:

Religion:

Occupation:

Address:

MRD:

Telephone no (if any):

Date of Examination:

B. CHIEF COMPLAINTS

C. HISTORY OF PRESENT ILLNESS

D. HISTORY OF PAST ILLNESS

Any GI surgery

Peptic ulcer disease

Malignancy

E. PERSONAL HISTORY

1. Smoking
2. Alcohol
3. Opium
4. Injectable drug use
5. Others

F. FAMILY HISTORY, if any

G. GENERAL SURVEY:

1. Weight
2. Height
3. BMI
4. BP
5. Others

H. LABORATORY INVESTIGATION

1) CBC
2) Absolute eosinophil count
3) RBS/FBS
4) RFT
5) TSH
6) LFT
7) ESR
8) CRP
9) ULTRASOUND ABDOMEN
10) ECG
11) CHEST XRAY
12) Stool for H. Pylori

I. ESOPHAGOGASTRODUODENOSCOPY and RUT

J. DUODENAL BIOPSY

K. SHORT FORM NEPEAN DYSPEPSIA INDEX

INFORMED PATIENT CONSENT

Date: _____

I……..D/o, W/o, S/o……. ……am willing to participate in the study **"ASSOCIATION OF FUNCTIONAL DYSPEPSIA WITH DUODENAL EOSINOPHILIA: A PROSPECTIVE CASE CONTROL STUDY"** The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

……. ……
Signature of the Witness Signature of the patient

Name:

Signature of Candidate **Signature of Supervisor**

मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____

निवासी _____

इस शोधकार्य **"ASSOCIATION OF FUNCTIONAL DYSPEPSIA WITH DUODENAL EOSINOPHILIA: A PROSPECTIVE CASE CONTROL STUDY"** में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

_____ _____

गवाह के हस्ताक्षर रोगी के हस्ताक्षर/अंगूठे का निशान

नाम: नाम:

_____

अन्वेषक केहस्ताक्षर

_____

विद्यार्थी के हस्ताक्षर

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ _____

“ASSOCIATION OF FUNCTIONAL DYSPEPSIA WITH DUODENAL EOSINOPHILIA: A PROSPECTIVE CASE CONTROL STUDY” ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ। ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕ ਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

_____ _____

ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____ ਮਿਤੀ_____

ਪਤਾ _____ ਪਤਾ _____

_____

ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

_____

ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ